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Role of Disulfide Bonds in Structural Stability and Flexibility of Cuticle-degrading Proteases from Nematophagous Fungi—A Molecular Dynamics Simulation Study

Lian-Ming Liang[1]
Ke-Qin Zhang[1]
Shu-Qun Liu[1,2]*

[1] Laboratory for Conservation and Utilization of Bio-Resources & Key Laboratory for Microbial Resources of the Ministry of Education, Yunnan University, Kunming 650091, P. R. China

[2] Sino-Dutch Biomedial and Information Engineering School, Northeastern University, Shenyang 110003, P. R. China

*shuqunliu@gmail.com

It has been shown that disulfide bonds play an important role in the stability of some proteins by an entropic effect (1), usually the globular proteins secreted to extracellular medium (2). Two cuticle-degrading proteases, Ver112 and PII, which were derived respectively from nematode-parasitic and nematode-trapping fungi, belong to the subtilisin family sharing relatively high sequence identity (45.7%). Ver112 is an alkaline protease and has two disulfide bonds, C35-C124 and C179-C250 (3); PII is a neutral protease and has no disulfide bond. Despite the minor structural difference between them (root mean square deviation (RMSD) is ~0.6 Å), Ver112 displays higher thermal stability and stronger nematicidal/catalytic activity than PII does (4).

In order to investigate how the disulfide bonds influence structural stability and flexibility of these two proteases, molecular dynamics simulations on their structures of wild-type and disulfide bond-disrupted mutant (Ver112_124C/A, Ver112_179C/A, and Ver112_124C/A_179C/A) were performed at temperatures 300 K and 400 K, respectively. Analyses of the geometrical properties along the 300 K MD trajectories indicate that PII has higher average values of C_α RMSD and solvent accessible surface area (SASA) while lower average values of number of native hydrogen bonds (NNH) and number of native contacts (NNC), suggesting a higher flexibility and less compact equilibrium structure of PII in comparison with Ver112. This may be caused by the lack of equivalent disulfide bonds in PII. The geometrical properties of Ver112 are similar on average to those of its three mutants during simulations at 300 K, while at 400 K the wild-type Ver112 presents more NHB and NNC and less SASA than its mutants suggesting that disulfide bonds contribute to the global stability of Ver112 at high temperature. Additionally, the stability of local structures within 5 Å of the two disulfide bonds C35-C124 and C179-C250 was also enhanced, as indicated by their increased RMSF and decreased NNC values upon disulfide bond breaking. Analyses of the average RMSF values of the S1 and S4 substrate-binding pockets show that upon disruption of C35-C124, RMSF of S1 pocket decreased by 21.2% while that of S4 pocket showed almost no change; upon disruption of C179-C250, the relatively large reduction in flexibility of both S1 and S4 pockets was observed; and the most pronounced reduction (30.7% and 17.2%) occurred when both disulfide bonds were broken. According to these results, we can conclude that i) the presence of disulfide bonds enhances not only the local but also the global stability of the protease, thus explaining the higher thermal stability of the alkaline protease Ver112 compared to that of the neutral protease PII; ii) the presence of disulfide bonds increases the flexibility of substrate-binding pockets located relatively far from disulfide bonds, thus explaining why alkaline proteases have higher substrate affinity (5, 6) and catalytic activity than neutral proteases.

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Thinking into Mechanism of Protein Folding and Molecular Binding

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Xing-Lai Ji[1,2]
Shu-Qun Liu[1,2]*

[1]Laboratory for Conservation and Utilization of Bio-Resources & Key Laboratory for Microbial Resources of the Ministry of Education, Yunnan University, Kunming 650091, P. R. China

[2] Sino-Dutch Biomedial and Information Engineering School, Northeastern University, Shenyang 110003, P. R. China

*shuqunliu@gmail.com

Protein folding and molecular binding provide the basis for life on earth. The native 3D structure of a protein is a prerequisite for its function; and the molecular binding is the fundamental principle of all biological processes (1). Therefore unraveling the mechanisms of protein folding and binding is fundamental to describing life at molecular level. Of particular interest is that protein folding and binding are similar processes because the only difference between them is the presence and absence of the chain connectivity. Among many models (such as diffusion-collision (2), hydrophobic collapse (3) and stoichiometry (4) models) proposed to describe the mechanism of these two processes, the "folding funnel" (5) model (Figure 1) is most widely accepted. In this model, protein folding can be viewed as going down the free energy hill through multiple parallel pathways towards the bottom of the funnel (6); and molecular binding can occur along rough free energy surface around the funnel bottom, especially for binding between flexible proteins/molecules. These are essentially thermodynamically controlled processes involving various types of driving forces, including the enthalpic contribution of noncovalent bond formations, entropic effects such as solvent release and burial of apolar surface area (hydrophobic effect), restrictions of degrees of freedom of protein/ligand, and loss of rotational and translational freedom of interacting partners. Briefly, these two processes, which are driven by a decrease in total Gibbs free energy (ΔG), are dictated by the mechanism of a delicate balance of the opposing effects of enthalpic (ΔH) and entropic (ΔS) contributions (equation 1).

$$\Delta \mathbf{G} = \Delta \mathbf{H} - \mathbf{T}\Delta \mathbf{S} \qquad 1$$

Here we emphasize that it is the thermodynamically driven subtle enthalpy-entropy compensation that leads to the global free energy minimum of the protein/ligand-solvent system (7), and that the specific inter-atomic interactions observed in the folded or complexed structure are to large extent the consequence of thermodynamic equilibrium but can not fully define the driving forces for folding and binding interactions.

Interestingly, we speculate that many other processes can be explained by thermodynamic enthalpy-entropy compensation, *i.e.,* the Yin and Yang balance in traditional Chinese medicine theory could correspond to the enthalpy and entropy compensation of the second law of thermodynamics; global warming can be considered as the consequence of excessive production of positive entropy (carbon dioxide) from chemically ordered fossil fuel, urging people to slow resource consumption to delay the inevitable death by entropy.

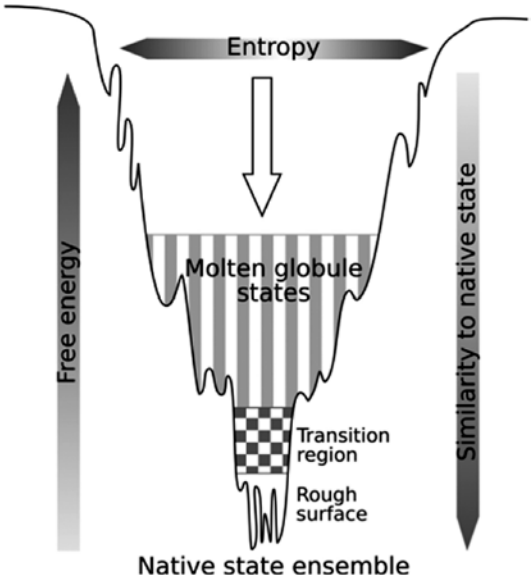

Figure 1: Schematic 2D funnel of protein folding and binding (modified from (6)).