STUDIES ON THE MECHANICAL STABILITY OF A PROTEIN BY SINGLE-MOLECULE ATOMIC FORCE MICROSCOPY

by

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ABSTRACT

Elastomeric proteins are subject to mechanical tensions under biological settings and possess mechanical properties that underlie the elasticity of natural adhesive, cell adhesion and muscle proteins. Single molecule atomic force microscopy has made it possible to directly probe the mechanical properties of elastomeric proteins and provides insights into the molecular design of elastomeric proteins. Combining the single molecule atomic force microscopy and protein engineering techniques allows us study the mechanical stability of proteins and develop methods to tune the mechanical stability. Mechanical tensions are also found in some nonmechanical proteins. Based on the results from single-molecule atomic force microscopy, nonmechanical protein of GB1 shows high mechanical stability that is comparable or superior to those of known elastomeric proteins. Here, we use a small protein, GB1, the B1 IgG binding domain of protein G from *Streptococcus*, as a model system to directly investigate the mechanical properties of GB1 mutants and loop mutants by using single-molecule AFM. Point mutations in proteins may disrupt the intermolecular interactions and affect the chemical and mechanical stability of the protein. Φ-value analysis together with single-molecule atomic force microscopy is used to probe the mechanical stability of the protein and gives a complete picture on how proteins are structured in the transition state during folding/unfolding event. Results from chapter 2 indicate that GF30L and GT53A mutation decrease the mechanical stability as well as accelerate the unfolding kinetics of GB1. This is due to the disruption of the hydrogen bond networking between the terminal β-strands or unraveled the hydrophobic interactions and side chain interactions, resulting in lower unfolding forces with Φ-values closer to one. Configurational entropy plays crucial roles in defining the thermodynamic stability as well as the folding/unfolding kinetics of proteins. Here, we directly probe the role of configurational entropy in the

mechanical unfolding kinetics and mechanical stability of proteins by using single molecule atomic force microscopy and protein engineering methods. Chapter 3 demonstrates that the mechanical stability of GB1 decreases as the number of inserted amino acid residues into loop 2 of GB1 increases. This result can be explained by the loss of configurational entropy upon closing an unstructured flexible loop using classical polymer theory, highlighting the important role of loop regions in the mechanical unfolding of proteins. The findings from these experiments are of critical importance towards engineering artificial elastomeric proteins with tailored nanomechanical properties.

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DEDICATION

This is dedicated to my family, my father, my mother, and my brother, for their constant supports and encouragements.

CO-AUTHORSHIP STATEMENT

The idea of Chapter 2 was identified and designed by Dr. Hongbin Li, Dr. Yi Cao and myself. I have performed the research and data analysis on wild-type GB1 GL7A, GT11A, GT16A, GT25A, GF30L, GD46 and GT53 mutants. Dr. Yi Cao performed the research and data analysis on GK28A, GK31A and GN35A. Manuscript preparation was done by Hui-Chuan Wang.

The idea of Chapter 3 was designed by Dr. Hongbin Li. Dr. Deepak Sharma and Dr. Meijia Wang performed the research and data analysis on GB1-L2 and GB1-L5. I carried out the research and analyzed the data on GB1-L24 and GB1-L46. Dr. Yi Cao performed the pulling speed experiment for all the GB1 loop mutants. The manuscript preparation was written by Dr. Hongbin Li.