Force Spectroscopy and Scanning Probe Microscopy

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Examining the Mechanical Properties of Copper Binding Azurin using Single Molecule Force Spectroscopy and Steered Molecular Dynamics

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The nature and properties of chemical bonds or interactions are of fundamental importance in understanding reactivity as well as biological functions of proteins. In particular, the metal-ligand bonds in metalloproteins are of special interest due to their partial covalent character and redox properties which might be sensitive to the protein environment. Hence, it is important to study and compare their mechanical strength and stability with those of covalent bonds. Here, we have investigated the mechanical properties of copper binding azurin using single molecule force spectroscopy (SMFS) and compared them with those of apo azurin. Azurin is a periplasmic protein which undergoes forced-unfolding during translocation from cytoplasm to periplasm. Our SMFS pulling studies on heptamer of azurin have revealed that holo azurin (copper bound protein) as well as its apo form unfold via an intermediate, which is mechanically weaker than the native state. We observed that the unfolding force of holo azurin is slightly higher (~10 pN) than that of apo azurin. Steered molecular dynamic simulations (SMD) simulations on apo azurin showed that the unraveling is initiated from the C-terminus by the rupture of H-bonds between b8 and b2B strands. Solvent accessibility of the native disulfide bond of azurin in the X-ray structure and in MD simulation showed that only ~15% of its surface area is accessible to the external aqueous solvent. Indeed, we have found from our pulling experiments that the native disulfide bond is inaccessible and resistant to chemical reduction by DTT and b-mercapto-ethanol. However, the disulfide bond gets exposed after the initial rupture of the native state and undergoes mechano-chemical reduction in SMFS experiments.

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Resolving Individual Damage Sites in DNA with AFM using Reengineered Repair Proteins

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UV irradiation can produce widespread and chemically heterogeneous damage that can accumulate to cause diseases, most notably melanoma and other types of cancers. The ability to identify, map and discriminate discrete damage sites on single DNA molecules can provide new diagnostics or insights into the progression and onset of precancerous conditions and melanoma. Atomic Force Microscopy (AFM) provides an ideal method to map individual damage locations because it produces real space images of single DNA molecules. However, UV-induced damage and many damage-binding proteins that could be used to label these sites are not directly resolvable by AFM. Here we have reengineered DNA damage proteins at the genetic level to be observable by AFM imaging when bound to damaged DNA. Using T4 Endonuclease V, a pyrimidine dimer-specific base excision repair protein too small to imaged by AFM, as a model UV damage repair protein we introduced a number of discrete I27 domains at the C-terminus, which sufficiently increases its size without interfering with native activity. Using these novel ''structural labels'', we are currently trying to visualize multiple damage locations simultaneously in both linear and genomic DNA via single molecule AFM imaging. These structural labels which can be adapted to a number of damage-binding proteins to target a variety of damage sites provide a potential methodology for single molecule diagnostics.

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Direct Observation of the Folding Trajectory of a Slipknotted Protein

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Over the last two decades, as a surprising feature for proteins, knotted/slipknotted topology has been found in certain proteins. Such knotted/slipknotted proteins need to overcome the topological difficulty in order to reach their functional native states. It is challenging to understand the folding mechanism of such proteins. AFV3-109 is a small alpha/beta protein containing a slipknot. In our previous studies, we have shown that stretching AFV3-109 from its N-C termini can readily lead to the unfolding of AFV3-109 and untying the slipknotted structure. However, it has been challenging to monitor the folding process of AFV3-109 from its unfolded conformation enroute to its slipknotted native state. Here use single molecule optical tweezers to investigate the folding process of AFV3-109. Using constant velocity mode, we found that unfolded AFV3-109, which was prepared by mechanically stretching from its both termini, can readily refold at a force of ~4 pN or lower to its native state with the slipknot conformation. The refolding of most AFV3-109 occurs in a sharp transition (with a time resolution of ~50 us), indicative of a two-state like transition without the accumulation of intermediate states. The apparent ease of such a folding into the slipknotted conformation for most AFV3019 suggests the topological barrier is not high for forming a protein slipknot under the experimental setting. Moreover, we observed that a small fraction of unfolded AFV3-109 molecules refold into the native state via a continuous folding trajectory, suggesting a more complex refolding pathway. These results open the avenue towards understanding of the folding mechanism of slipknotted and knotted proteins.

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Mutual a Domain Interactions in the Force Sensing Protein von Willebrand Factor (VWF)

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VWF is a glycoprotein that plays a central role in hemostasis. It is, amongst others, responsible for platelet adhesion at sites of injury via its A1 domain. The adjacent VWF domain A2 is unfolded under shear upon which it exposes a site cleaved by the protease ADAMTS13 to prevent thrombosis. It has been shown that the A1/platelet interaction is blocked by mutual A1/ A2 binding under low shear. Nevertheless, the exact shielding mechanism is not yet clarified. We therefore probed the interaction strength between the A1 and A2 domains and the unfolding behavior of A2 as an indicator of the shielding capacity utilizing single molecule force spectroscopy (SMFS). SMFS studies between the isolated domains A1 and A2 revealed specific recognition with a bond life-time of ~1.5s and forces between 50 and 140pN at loading rates ranging from 100 to 60000pN/s. To elucidate the interplay between dissociation of the A1-A2 complex and A2 domain unfolding, we analyzed the elongation of the complex prior to dissociation. In most cases, the measured elongation values (~28nm) were substantially lower than the extension of a fully-unfolded A2 domain (~80nm). Additionally, we used a disulfide bridged A2 domain mutant ([A2]). The introduction of the disulfide bond obstructs the mechanical unfolding of this domain. Similar complex elongations were observed for both, the A1/A2 and the A1/[A2] interactions. Thus our data indicate, if at all, only a small extent of A2 unfolding before dissociation from A1. Overall, these findings suggest that the platelet binding site in domain A1 is made accessible by the dissociation of domain A1 from A2 and that domain A2 remains largely folded during this process, which keeps it protected against cleavage and degradation.

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Surprising Force-Dependent Unfolding of Titin Immunoglobulin Domain Revealed by Magnetic Tweezers

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Titin is a giant muscle protein which helps to maintain the integrity of sarcomere. The folded or unfolded states of immunoglobulin domain in titin

regulate the flexibility of muscle. How physiological force affects the unfolding rate is important to the function of titin. Using ultra-stable magnetic tweezers, we report systematic study of force-dependent unfolding dynamics of titin I27 immunoglobulin domain. Also the effect of low concentration of denaturant might be reported.