Linking mechanochemistry with protein folding with single bond resolution

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Abstract

The nanomechanical properties of elastomeric proteins determine the elasticity of a variety of tissues. Post-translational modifications (PTMs) have recently emerged as a useful tactic to regulate protein nanomechanics. In particular, the presence of covalent disulfide bonds, arguably the most relevant PTM with a significant mechanical role, is a widespread natural strategy to regulate protein extensibility and enhance protein stiffness. The prevalent in-vivo strategy to form disulfide bonds requires the presence of dedicated enzymes. Here we propose two alternative chemical routes to promote non-enzymatic oxidative protein folding through the reactivity of protein based chemical modifications. Using single-molecule force-clamp spectroscopy and mass spectrometry, we first captured the reactivity of an individual sulfenic acid, a PTM that functions as a key sensor of oxidative stress, when embedded within the core of a single Ig domain of the titin protein. Our results demonstrated that sulfenic acid is a crucial short-lived intermediate that dictates the protein's fate in a conformation-dependent manner. When exposed to the solution, sulfenic acid rapidly undergoes further chemical modification, leading to irreversible protein misfolding; when cryptic in the protein's microenvironment, it readily condenses with a neighbouring thiol to create a protective disulfide bond, which assists the functional folding of the protein. A second, alternative method to induce disulfide reformation occurs via disulfide isomerization of naturally occurring small thiols. Our single molecule approach, complemented with DFT calculations revealed that subtle changes in the chemical structure of a transient mixed-disulfide intermediate adduct between a protein cysteine and an attacking low molecular-weight thiol have a dramatic effect on the protein's mechanical stability. Combined, these chemistry-based mechanisms for non-

enzymatic oxidative folding provide a plausible explanation for redox-modulated stiffness of proteins that are physiologically exposed to mechanical forces, such as cardiac titin.