Identifying and Quantifying Kinetic Stability in Proteins via Electrophoresis

by

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ABSTRACT

Unlike most natural proteins, which are marginally stable, some hyperstable proteins are characterized by possessing a high-energy barrier towards unfolding that virtually traps them in their native state. These proteins are known as kinetically stable proteins (KSPs) by having the rare property of kinetic stability. KSPs are usually resistant to proteolysis, detergents, and have longer half-life under extreme conditions. The loss of protein kinetic stability could lead to protein misfolding and aggregation with pathological consequences. KSPs also find potential applications in various industrial fields, where harsh environments, extended storage or the presence of chemicals are required. However, the structural basis and the physiological and pathological implications of kinetic stability remain poorly understood due to the lack of simple methods to identify KSPs. A diagonal two-dimensional (D2D) sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) assay to identify KSPs in mixtures was previously developed based on the correlation between a protein's resistance to SDS resistance and its kinetic stability. Xia, *et al*, applied the assay to the cell lysate of *E.coli* and after proteomics analysis they identified 50 putative KSPs. This thesis expands the D2D SDS-PAGE assay to two other bacteria, *Vibrio cholerae* and *Bacillus subtilis*. Proteomics analysis involving MALDI-TOF/TOF led to the identification of seventeen and fourteen KSPs in *Vibrio cholerae* and *Bacillus subtilis*, respectively. The function and structure of those identified KSPs were analyzed and the results were compared with those of *E.coli*. Similar to KSPs in *E.coli*, KSPs in *Vibrio cholerae* and *Bacillus subtilis* have a functional bias toward enzymes and a structural bias toward complex structure, such as mixed α/β structure and oligomeric structures. No monomeric all-α helical KSP was found in either bacterium. The similar biases toward or against kinetic stability in the three bacteria shed some light on the evolutionary selection of kinetic stability in bacteria. This thesis also describes the development of two methods to identify KSPs. One of them is a 1-dimensional (1D) SDS-PAGE assay to estimate the kinetic stability of a protein. This method involves the incubation of KSPs in SDS buffer for different time periods at elevated temperatures, and then analyzing each time point sample by 1D SDS-PAGE, followed by quantitative analysis of the gel data. Eight KSPs, inorganic pyrophosphatase (PPase), lectin from

mushroom, β-glucosidase (BGL), glucose oxidase (GO), streptavidin (SVD), transthyretin (TTR), superoxide dismutase (SOD) and α-amylase (AMY), were subject to this assay and their kinetic stability was quantified. Compared to the D2D SDS-PAGE method, which only provides qualitative information about kinetic stability, this 1D SDS-PAGE assay not only provides quantitative information on kinetic stability, but also ranks the kinetic stability among KSPs. In addition, this assay is flexible because it has adjustable parameters, such as detergent type, incubation temperature, and time. The other method developed in this thesis is a gel-free capillary electrophoresis (CE) assay for identifying KSPs. CE was applied to two sets of proteins: four non-KSPs and four KSPs. The electropherograms of the two sets showed distinct migration time and peak pattern. The non-KSP set migrated at a similar time (13.4-14.2min) because of their similar charge-to-mass ratio. In contrast, the KSP set migrated at different times according to their characteristic charge-to-mass ratios. CE is simple, fast and automated. It excludes the effects from sizes of the protein and of gel pore, which results in more sensitive and efficient separation. The study described in this thesis not only provides diverse methods to identify and quantify kinetic stability in proteins, but also leads a better understanding of the structural and biological basis of kinetic stability.