Identifying Kinetically Stable Proteins via D2D SDS-PAGE

by

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ABSTRACT

Most proteins are in equilibrium with partially and globally unfolded conformations. In contrast, kinetically stable proteins (KSPs) are trapped by an energy barrier in a specific state, unable to transiently sample other conformations. It appears that kinetic stability (KS) is a feature used by nature to allow proteins to be resistant to harsh conditions and to protect the structure of proteins that are prone to misfolding and aberrant self-assembly. This intriguing property may have tremendous biological significance, beyond what is currently appreciated. Furthermore, KS should be a useful property for proteins with potential industrial applications that require high temperature, extended storage, or the presence of chemical (e.g. detergents), that would cause normal proteins to lose their structure and function. However, few KSPs have been identified to date due to the lack of simple experimental methods to identify this property. Previous work in the Colón lab established a correlation between KS and a protein's resistance to the denaturing detergent sodium dodecyl sulfate (SDS). Based on this correlation, this thesis describes the development of a diagonal two-dimensional (D2D) SDS-polyacrylamide gel electrophoresis (PAGE) assay to identify KSPs in complex mixtures of proteins. This method was also coupled with proteomics analysis, such as LC-MS/MS or MALDI-TOF/TOF, and resulted in the identification of 50 non-redundant putative KSPs. Functional and structural analyses of a subset of these proteins with known 3D structure revealed a potential bias of enzymes and mixed secondary structures (α/β structure) towards KS. Interestingly, no monomeric all-α-helical protein was identified as a KSP, suggesting that structural complexity may be a pre-requisite for KS. The D2D SDS-PAGE method was also applied to a variety of organisms from eukaryotes (yeast, mushroom, kernel of maize, Raji and Jurkat), to prokaryotes (*E. coli*, *T. thermophilus* and *Vibrio cholerae*) to human fluids (plasma and cerebrospinal fluid). Remarkably, it was observed that the number of KSPs was significantly lower in eukaryotes than in prokaryotes, suggesting that KS is a property that is

more compatible with prokaryotic organisms, perhaps due to their less sophisticated biological components and higher tendency to live in harsher environments. In contrast, the more intricate and highly regulated eukaryotic organism may have less need or compatibility for KSPs. When applied to human plasma, preliminary proteomics analysis revealed 10 KSPs. Among them, two inflammation-related proteins, complement component 3 and haptoglobin, were observed to aggregate more severely in elderly individuals. Another protein identified was transthyretin (TTR), which is linked to familial amyloid polyneuropathy (FAP). It has been shown that the mutation-induced lost of TTR KS plays a causative role in FAP. Interestingly, D2D SDS-PAGE of *E. coli* overexpressing pathogenic A25T TTR mutant revealed a different migration compared to WT TTR, consistent with its less KS. Therefore, these examples with proteins present in human plasma suggest that the D2D SDS-PAGE assay has the potential to serve as a tool for biomarker discovery and diagnostic applications. Thus, the studies describe here show that this novel D2D SDS-PAGE method could have widespread biological, biomedical, and industrial application, and thereby eventually lead to a better understanding of the structural basis and biological significance of protein kinetic stability.