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Do Prokaryotes Have More Kinetically Stable Proteins Than Eukaryotic Organisms?†

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ABSTRACT: Upon folding, some proteins become conformationally trapped, presumably to protect against aggregation or premature degradation. To probe the occurrence of this property, known as kinetic stability, we used a diagonal two-dimensional sodium dodecyl sulfate−polyacrylamide gel electrophoresis method to probe biologically diverse organisms. The results show that kinetic stability is prevalent in prokaryotes, especially thermophiles, but uncommon in eukaryotic organisms, thereby suggesting that this property might be crucial for the adaptation and survival of less complex prokaryotic organisms.

Despite their rich functional diversity, most natural proteins are marginally stable, suggesting that nature can tolerate and might even favor the minor stability of proteins in favor of functional and organism complexity. However, some proteins are hyperstable, as demonstrated by their resistance to proteolysis and detergents and their long half-life under extreme conditions. These proteins are characterized by having a high energy barrier toward unfolding that virtually traps them in their native state (*1*, *2*). This rare property of proteins, known as "kinetic stability" (KS), appears to be a strategy used by "mother nature" to protect certain proteins against aggregation, as well as premature degradation (*3*, *4*). Although the structural basis and the physiological and pathological implications of KS remain poorly understood, it has been demonstrated that the loss of protein kinetic stability could lead to protein misfolding and aggregation with pathological consequences (*2*, *5*). Thus, it would be intriguing to determine how many proteins in nature might be kinetically stable and to explore the potential biological and pathological implications of this property. On the basis of an observed correlation between kinetic stability and a protein's resistance to the detergent sodium dodecyl sulfate (*6*), we recently applied a diagonal two-dimensional (D2D) sodium dodecyl sulfate−polyacrylamide gel electrophoresis method for the proteome-level identification of kinetically stable proteins (KSPs) (Figure 1 and Supporting Information) (*7*). Application of D2D SDS−PAGE to the lysate of *Escherichia coli* combined with subsequent proteomics analysis resulted in the identification of 50 KSPs (*7*). To begin probing the pervasiveness and biological implications of KSPs, we applied the D2D SDS−PAGE assay to the cell lysates of various prokaryotic and eukaryotic organisms. It should be noted that although proteins will have a range of kinetic stability, D2D SDS−PAGE allows only the identification of those proteins, which by their SDS resistance, clearly possess high kinetic stability. Within this context, we describe as kinetically stable only those proteins that are SDS-resistant.

The cell lysates of thermophilic and mesophilic prokaryotic microbes, as well as eukaryotic organisms, were analyzed by D2D SDS−PAGE to determine the relative abundance of KSPs in diverse life forms across different kingdoms (*7*). The thermophilic microorganisms analyzed were the bacteria *Thermus thermophilus* and *Thermus aquaticus* and the archaea *Sulfolobus acidocaldarius*, which grow at optimal temperatures of 65, 70, and 80 °C, respectively. *S. acidocaldarius* also requires acidic conditions (pH 3.0). These thermophiles exhibited the highest abundance of SDS-resistant (i.e., KSPs) proteins (Figure 2a and Supporting Information). Interestingly, most of the KSPs in the archaea *S. acidocaldarius* migrated at the far-left area of the gel, suggesting that many of the KSPs are involved in high-molecular weight complexes. The mesophilic bacteria probed in this study were *E. coli* (*7*), *Vibrio cholerae*, and *Bacillus subtilis*, which grow optimally at 30−37 °C. These mesophiles exhibited significant variation in the number of KSPs present, but in general, the number of KSPs was significantly less than for the thermophiles, especially in the top left quadrant of the gel where the higher-molecular weight proteins and complexes migrate (Figure 2b). Many eukaryotic organisms were available for analysis, and three widely different organisms from separate kingdoms were selected for this study, including *Saccharomyces cerevisiae*, maize, and *Tetrahymena thermophila*. Remarkably, the 2D gels of all the eukaryotic organisms tested exhibited very few, if any, KSPs (Figure 2c). Thus, there was a clear difference in the abundance of KSPs in different organisms, with thermophiles and prokaryotes having more KSPs than mesophiles and eukaryotes, respectively.

The results of this study have implications for the evolution of protein stability and function. Kinetic stability was likely a critically important property of proteins for the adaptation and survival of microbial organisms, which in the absence of cellular subcompartments and advanced defense and metabolic systems, had to rely on the resilience of its protein "warriors" to resist the harsh outside world. The endurance of KSPs against harsh environmental conditions could allow organisms to preserve nutrients and energy that would otherwise be expended in continual protein degradation and synthesis. In contrast, the apparent minimal abundance of KSPs in eukaryotic organisms implies an evolutionary compromise of KS in favor of more complex cellular defense, function, and regulation. Interestingly, the sophisticated protein quality control machinery, the presence of organelles, and the usually milder environment of eukaryotic organisms might explain why the ubiquitous presence of KSPs does not seem to be a requirement of complex life forms. Furthermore, it appears likely that the robust physical properties of KSPs might not be generally compatible with the ever-present

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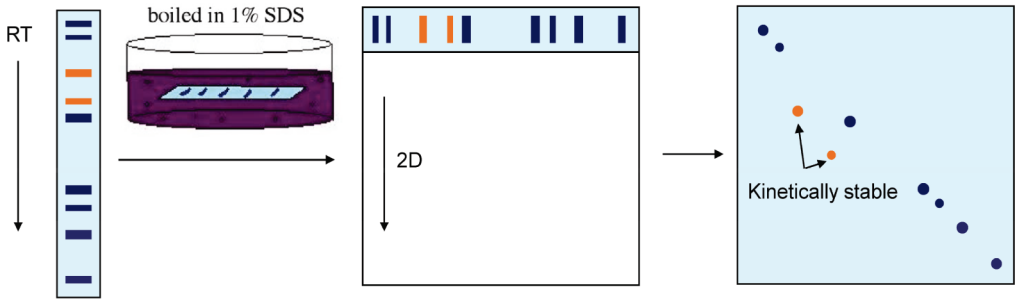

FIGURE 1: Illustration of the D2D SDS−PAGE method for detecting kinetically stable proteins (KSPs) in cell lysates. Because KSPs migrate less on SDS−PAGE when the sample is not heated because of their resistance to SDS, the essence of this assay is the combination of nonheating (one-dimensional run) and heating (two-dimensional) steps within the same gel experiment. One-dimensional SDS−PAGE is performed at room temperature followed by excision of the relevant gel strip, which is then incubated in boiling 1% (w/v) SDS buffer for ~10 min. The strip is placed on top of a gel plate before polymerization of a new gel, and a two-dimensional SDS−PAGE separation is performed. After being stained, the gel reveals a diagonal band resulting from the equal migration of non-KSPs (blue spots) in both dimensions. KSPs (orange spots) migrate less in the first dimension and therefore migrate to the left of the gel diagonal.

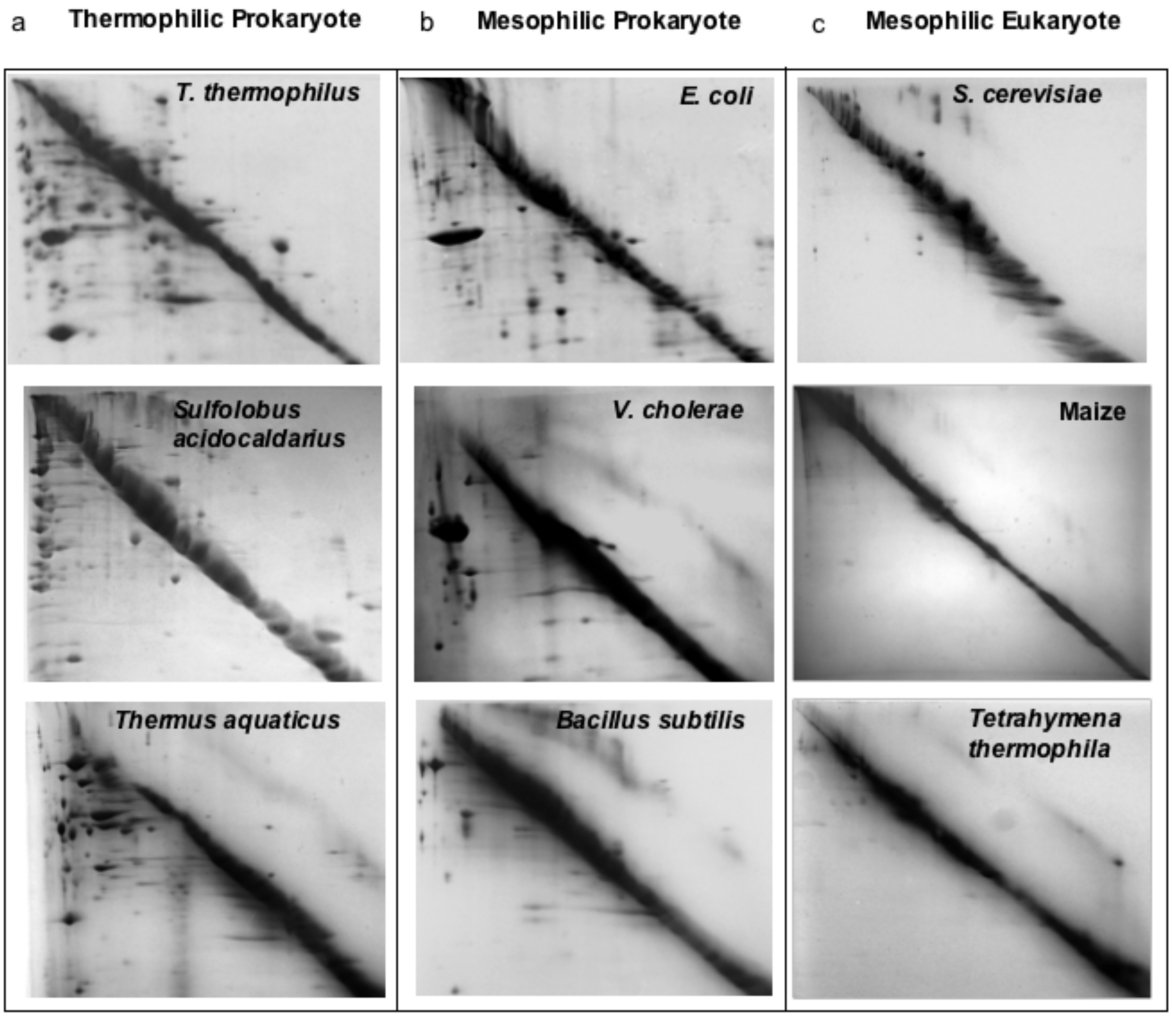

FIGURE 2: D2D SDS−PAGE of the lysate of various organisms to probe the extent of kinetically stable proteins present. (a) Thermophilic prokaryotes (*T. thermophilus*, *S. acidoldarius*, and *T. aquaticus*) exhibited significantly more spots migrating to the left of the diagonal than (b) mesophilic prokaryotes (*E. coli*, *V. cholerae*, and *B. subtilis*). (c) Mesophilic eukaryotes (*Sa. cerevisiae*, maize, and *Te. thermophila*) showed the fewest number of KSPs spots. Because of differences in background staining, the pictures were slightly enhanced by Microsoft office picture manager through linear adjustment of contrast, brightness, and color applied to the entire image in each case.

post-translational modification, translocation, and regulatory processes of eukaryotic organisms. Nevertheless, kinetic stability might still have some important roles in eukaryotic organisms, such as the suppression of misfolding and aggregation of certain proteins. Notably, the loss of kinetic stability of the proteins transthyretin, Cu/Zn superoxide dismutase, and phenylalanine hydroxylase has been linked to the diseases familial amyloid polyneuropathy, familial amyotrophic lateral sclerosis, and phenylketoenuria, respectively (*5*, *8*, *9*). Despite the relatively low abundance of KSPs in eukaryotes, many roles for kinetic stability are likely to exist in these complex organisms. Analogously, the presence today of many KSPs in prokaryotes, especially

thermophiles, suggests that this property is still required for the survival of these structurally simpler organisms, which have developed strategies, including the presence of KSPs to adapt and proliferate in extreme environments. Intriguing questions remain, such as what these proteins are and what their specific role in determining the resilience of bacteria to harsh conditions might be.

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SUPPORTING INFORMATION AVAILABLE

Materials and methods. This material is available free of charge via the Internet at http://pubs.acs.org.

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