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compared to hearts from wild-type transgenic mice. Protein levels of the 19S regulator subunit Rpt1 were increased in the hearts of 3-month-old I79N mice and decreased in the hearts of 1-year-old R278C mice. Expression of PKC alpha, an important regulator of cardiac contractility, was decreased in R278C mice at 1 year. Activity of the lysosomal protease cathepsin L was increased in 1-year-old I79N mice, indicating disruption of another major proteolytic system in these hearts. Quantitative real-time PCR of the genes involved in the ubiquitination pathway (E1, E2, and E3 enzymes) showed that transcript levels were increased or decreased for 25 genes in I79N mice and three genes in R278C mice. These data demonstrate that the UPS is affected at the gene expression and protein level in R278C and I79N mice, indicating that changes in the UPS may be involved in FHC.

Platform: Protein Folding & Stability I

965-Plat

Single-Molecule FRET Shows Folding Transition Path Time for All-Alpha Protein Slowed by Internal Friction

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The transition-path is the tiny fraction of an equilibrium, single-molecule trajectory when a transition occurs between two states. The importance of the transition-path in protein folding is that it contains all the mechanistic information on how a protein folds and unfolds. However, a transition-path has never been observed experimentally for any molecular system in the condensed phase because it is too fast to measure. Even determining the average transition-path time, $\langle t_{TP} \rangle$, is challenging. Previously, we determined $\langle t_{TP} \rangle$~2μs for the all-β protein, FBP28 WW-domain ($1/k_F$=100μs) and an upper bound of $\langle t_{TP} \rangle$~10μs for the much slower αβ-protein GB1 ($1/k_F$ =1s) by employing the Gopich-Szabo maximum likelihood analysis of photon trajectories in single-molecule FRET experiments and a kinetic model in which the lifetime of an additional state in a one-step discretization of the transition path corresponds to $\langle t_{TP} \rangle$ (Chung *et al*., *Science* 2012). Surprisingly, the $\langle t_{TP} \rangle$s for the two proteins differ by <5-fold, while the folding rates differ by ~10,000-fold. Even more surprising is that this result can be explained by the theory for diffusion of a Brownian particle over a barrier on a one-dimensional free-energy surface, which predicts $\langle t_{TP} \rangle$ to be insensitive to the barrier height but to scale as $1/D^*$, the diffusion coefficient at the barrier top, i.e. $\langle t_{TP} \rangle \propto \ln(3\beta\Delta G^*)/D^*$. Maximum likelihood analysis of photon trajectories for α_3D, an all-α protein ($1/k_F$=2ms), reveals an additional-state lifetime of 12μs. While the folding time for all-β proteins scales linearly with the solvent viscosity, like other all-α proteins, the folding time of α_3D scales sub-linearly with viscosity ($1/k_F \sim \eta^{1/2}$), as does the 12μs lifetime. These results indicate that this additional lifetime corresponds to $\langle t_{TP} \rangle$, slowed compared to the WW-domain by a larger contribution of internal friction to D^*.

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Insight into the Molecular Origins of the Internal Friction in Unfolded Proteins

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Protein folding and dynamics can be modeled as a diffusional process on a low-dimensional free energy surface. Contributions to this dissipative process can be separated into solvent dependent or wet friction and internal or dry friction, where frictional effects are due to the chain itself. Experimental evidence and polymer models (Soranno, A. et al., Proc. Natl. Acad. Sci. USA, 2012) have shown that the internal friction is an additive contribution to the reconfiguration time of unfolded proteins. Despite recent advances, the molecular origins of these effects have remained largely elusive. Using extensive all-atom molecular dynamics simulations we studied the dynamics of the unfolded cold-shock protein (CSP) from Thermotoga maritima at different solvent viscosities and at different denaturant concentrations (including the denaturant free case). Reconfiguration times obtained from MD simulations are consistent with experimental results. Also, in agreement with experimental results, simulations done at different denaturant concentrations suggest that the internal friction contribution correlates with the compactness of the unfolded protein. We used diffusion map analysis to characterize the slow diffusive variables and cluster the states sampled during each simulation. We systematically analyzed the reconfiguration dynamics of relevant structural features such as hydrogen bonds formation (native and non-native) and dihedral angle rotations. By defining the relaxation timescale of these structural features we were able to identify their contributions into the internal friction in the unfolded state. These results have important implications for the folding kinetics of proteins especially when considering protein folding in the context of the denaturant-free environment of a living cell. Under these conditions, the internal friction contribution may be dominant in the folding process.

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Two-Dimensional Infrared Spectroscopy as a Probe of Protein Folding: Bridging the Gap between Experiment and Simulation

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Two-dimensional infrared (2DIR) spectroscopy is a newly-developed experimental technique that measures protein structure and dynamics in solution with subpicosecond time resolution. Amide-I vibrations, consisting mainly of backbone C=O stretching modes, contain a wealth of structural information. Two-dimensional spectroscopy offers enhanced structural sensitivity by spreading the spectral information onto two frequency axes. Through a combination of temperature-jump 2DIR spectroscopy, isotope labeling, and Markov state models derived from molecular dynamics simulations, we develop a new method which can directly probe the structural rearrangements on timescales from nanoseconds to milliseconds. Markov state models provide an intuitive interpretation of the protein folding process while retaining much of the structural heterogeneity and diversity of folding pathways.
The unfolding mechanism of a 39-residue α/β mini protein, NTL9, a two-state folder, is studied on timescales from 100 ns to 50 milliseconds. Transient 2DIR reveal a rapid sub-100 ns response that is attributed to weakening of the hydrogen-bonds, followed by unraveling of the beta sheet. The more stable helix is seen to denature on the 150 microsecond timescale. Experimental data is interpreted in the context of the recently-available Markov state model of NTL9. Simulated 2DIR spectra are generated for the structural ensemble, and are observed to be in great agreement with the temperature-jump 2DIR experiments. The results provide an elegant illustration of how a combination of cutting-edge experiments and state-of-the-art simulations gives new insights into the complex mechanism of protein folding.

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Protein Folding Studied by Very Fast Pressure Drops

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Temperature jump experiments have been used to study the kinetics of protein folding for over 20 years. These experiments have driven our understanding of protein folding and our attempts to simulate protein dynamics. It may be easier, however, to model and understand the effect of pressure perturbations on proteins because pressure affects biomolecular structure only through density changes, not through changes of thermal energy or solvent composition. In 2009 our lab was the first to report a pressure drop apparatus, which could be used to change the pressure of a protein solution from 2500 atm to 1 atm in less than a microsecond, causing the proteins to fold. We have recently used that instrument to study the refolding of a mutant of a protein called lambda repressor fragment 6-85 ($\lambda^*_{6\text{-}85}$). $\lambda^*_{6\text{-}85}$ is one of the fastest folding proteins ever discovered, with all of its known mutants folding in less than 50 μs. Surprisingly, we have observed a slow (~1 ms) phase in the refolding of a mutant of $\lambda^*_{6\text{-}85}$ initiated by a fast pressure drop. The emergence of this slow timescale highlights the necessity of expanding our toolbox for studying the dynamics of protein folding, as it may be that only certain conformations are populated by manipulation of a single thermodynamic variable.

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The Effects of Crowding and Osmolytes on the Temperature-Pressure Stability and Intermolecular Interactions of Proteins

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Mechanisms have evolved in nature that allow living organisms to deal with extreme environmental conditions by producing organic osmolytes. For example, certain marine organisms living in the deep sea have evolved a surprising simple mechanism to counteract the deleterious effects of the cellular waste product urea and of hydrostatic pressure by trimethylammonium *N*-oxide (TMAO). Hence, we investigated the effect of pressure on the structure and intermolecular interactions of dense lysozyme solutions in urea-TMAO mixtures using small-angle X-ray scattering in combination with a liquid-state theoretical approach based on the DLVO potential which accounts for repulsive and attractive interactions between the protein molecules. Supplementary thermodynamic information was obtained by employing calorimetric techniques, densitometry and ultrasound velocimetry. We show that the particular structural