comparison to high-pressure crystal structures of L99A T4 lysozyme [Collins, et al. (2005), PNAS 102, 16668-16671], pressure denaturation of the structurally similar L99A and L99G/E108V mutants was studied at neutral pH. The pressure-denatured state at neutral pH is even more compact than at low pH, and the small volume changes associated with denaturation suggest that the preferential filling of large cavities results in a compact, pressure-denatured state. These results confirm that pressure denaturation is characteristically distinct from thermal or chemical denaturation.

1699-Pos Board B543

A Peek into Tropomyosin Unfolding on the Actin Filament

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Tropomyosin (TM) is a coiled-coil along its length with subtle variations in structure that allow interactions with actin and other proteins. Actin binding globally stabilizes tropomyosin. Here we ask, ''Does TM unfold in the presence of F-actin as a single unit or in multiple blocks?'' We hypothesize that functional binding sites unfold prior to or during dissociation from actin, preceding chain separation. We refer to the seven periodic repeats (Phillips, 1986) as P1-P7. We monitored local unfolding and chain dissociation by fluorescence of pyrenylated TM (Ishii & Lehrer, 1980). We combined fluorescence with light scattering and DSC (Levitsky et al., 2000) to monitor TM unfolding and dissociation from F-actin. We investigated the relationship of specific regions of the molecule to unfolding and dissociation of the entire molecule from F-actin by pyrenylation of Cys190 (in P5), and in TMs engineered to have a single Cys analogous to that of Cys190 in P2 and P3 (''controls''). We previously reported that the destabilizing Ala cluster in P5 is required for its participation as a ''strong'' binding site (Singh and Hitchcock-DeGregori, 2006). An Ala cluster was introduced in P2 or P3 to mimic P5. Analysis of the ''controls'' and mutants showed (1) binding to actin stabilizes all TM variants reflected by the Tm of excimer formation, and (2) that locally destabilized regions in P2, P3, and P5 unfold prior to or during dissociation from F-actin. Initial unfolding of the P2 and P3 regions is distinct, but overlaps at higher temperatures indicating the unfolding of these regions does not occur in a single block but in multiple overlapping blocks. This, and previous work, suggests that regions of TM involved in binding actin have a poorly packed interface and are locally stabilized upon binding. Supported by NIH.

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Characterizing the Fitness Effects of Mutations in the Yeast *URA3* Gene

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The central goal of this project is to study the mechanisms by which changes in gene sequence affect the level of function of the associated protein and therefore, fitness of the organism that carries a particular allele of a gene. In order to study this process, we performed random mutagenesis of the yeast *URA3* gene and are currently assaying the mutated sequences for their function by competing yeast strains that have different alleles of the *URA3* gene. The results from such a study would allow us to address some long standing issues in genetics–for example, the tolerance of proteins to amino acid substitutions, and secondly, the relative importance of the interaction between mutations as compared to the additive effects of mutations on the fitness of the gene. We are also interested in studying sequence evolution from a computational perspective, and more specifically, evaluating how much information about the structural constraints of a protein can be extracted from many homologous sequences. Using a statistical coupling analysis on a multiple sequence alignment of 620 *URA3* sequences from different organisms, we have identified two large co-evolving networks of residues in the enzyme. Our preliminary results indicate that statistical coupling analysis is a powerful tool for identifying mutations that are likely to cause fitness effects.

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Cation-pi Interactions Contribute Significantly To The Stability Of The D2 Domain Of Fibroblast Growth Factor Receptor

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Fibroblast growth factors (FGFs) are ~ 16 kDa heparin binding proteins that regulate key cellular processes such as angiogenesis, differentiation, morphogenesis, wound healing and tumor growth. FGFRs consist of three extracellular ligand binding domains (D1, D2, D3), a single transmembrane helix, and cytoplasmic tyrosine kinase domain. Cell surface-bound HSPGs (heparan sulfate proteoglycans) supported dimerization or polymerization of the FGFRs are thought to be required to activate the signaling pathway. The D2 domain is suggested to bind with both HSPGs and FGFs to form a ternary complex. Xray and NMR solution structures of the D2 domain have been analyzed using the CAPTURE cation-pi program. The CAPTURE program indicates cation-pi interactions between residues Y10:R7 (Xray), W46:R58 (NMR) and possibly F92:K6 (Xray). Biophysical characterization of the mutants at each cation and pi pair, identified by CAPTURE, shows a significant destabilization resulting from the Y10A, W46A and R58E mutations. Results from differential scanning calorimetry show a reduction in melting temperature by 10-14 °C for Y10A, W46 and R58 mutants of D2. The reduction in the stability of the D2 domain is corroborated by results of ANS binding, thermal denaturation and a limited trypsin digestion experiments. The HSQC of D2 Y10A shows limited chemical shift perturbation of residues in the vicinity of the mutation site. The W46A and R58E mutations show significant ^{1}H-^{15}N chemical shift perturbations in their HSQC spectra. The results obtained in this study show that cation-pi interactions contribute significantly to the thermodynamic stability of proteins. In addition, our results indicate that cation-pi predictions made on solution NMR structures are more reliable than those predicted based on crystal structures.

1702-Pos Board B546

Contributions of Tyrosine Residues to the Stability of Human γD-Crystallin

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The lens protein human γD-crystallin (HγD-Crys) belongs to the βγ-crystallin family and exhibits two homologous Greek key domains, each containing eight β-strands. HγD-Crys must remain soluble and folded throughout the human lifetime. Aggregation of crystallins leads to cataract.

14 of the 173 amino acids in HγD-Crys are tyrosines. The ''tyrosine corner'' is a conserved structural element of the Greek key, which bridges β-strands, by hydrogen bond between tyrosine hydroxyl group and a backbone carboxyl group. Interacting tyrosine pairs at the turns of β-strands are also involved in an extensive aromatic network throughout HγD-Crys. These tyrosine corners and tyrosines pairs may be important in the mature stability and/or folding pathways.

Site-specific mutants of four of the relevant tyrosines to alanines or phenylalanines were constructed. All mutant proteins adopted a native-like conformation by circular dichroism (CD). Thus the tyrosine side chains do not appear to be essential in directing the β-sheet fold.

To assess stability, equilibrium unfolding/refolding experiments were performed in guanidine hydrochloride (GuHCl) at pH 7.0, 37°C. For the tyrosine corner mutants, Y62F and Y62A both had a destabilized N-terminal domain (N-td), but unaffected C-terminal domain (C-td), with increased population of the single folded domain intermediate. Y150F and Y150A had a destabilized C-td and showed a more cooperative folding process. The double mutant Y62F/Y150F had both N-td and C-td destabilized. These results indicated that the hydroxyl groups on tyrosine corners are important in the thermodynamic stability of HγD-Crys. For the tyrosine pair mutants, Y45A and Y50A both had a destabilized N-td, but unaffected C-td. In contrast, Y45F and Y50F had no significant difference in stability compared with the wildtype. Thus the tyrosine pairs contribute to the stability of mature HγD-Crys largely through their aromatic rings.

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The Energetics of the Denaturation of the C2A Domain of Synaptotagmin I

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Thermodynamic parameters capture the averaged contribution to a system's energetics. In the case of binding proteins, such as synaptotagmin I, the first step toward addressing how and where the energy is distributed within that protein is to ascertain the magnitude of the interactions within that protein. Our aim is to understand how binding information is conveyed throughout this protein during the role in plays in regulated exocytosis. While many detailed molecular approaches have identified putative regions where interactions occur, it is their energetics that dictates their response. Here, denaturation studies of the C2A domain of synaptotagmin I were carried out in conditions that are physiologically relevant to regulated exocytosis where the calcium ions and phospholipids were either present or absent. Denaturation data was collected using two techniques: differential scanning calorimetry (DSC) and lifetime fluorescence. A global analysis approach combining these data sets was used where the data was simultaneously fit to models derived from thermodynamic first principles.