1694-Pos Board B538
Thermal Adaptation Strategies used by TBP
Victor M. Capistran-Licea, Cesar Millan-Pacheco, **Nina Pastor**.
Universidad Autonoma del Estado de Morelos, Cuernavaca, Morelos, Mexico.
The TATA binding protein (TBP) is a monomeric transcription factor present in archaea and eukarya, in organisms adapted to temperatures spread over a 100°C range. Thermal adaptation implies a balance between stabilization and function, which entails keeping ligand selectivity and also the amplitude of conformational fluctuations within physiologically reasonable bounds. To explore relevant contributions to thermal adaptation of the TBP fold, we perturbed the native structures of six TBP variants coming from organisms with optimal growth temperatures between 10°C and 105°C, with molecular dynamics simulations at 273, 298, 323, 348 and 373K, and 1 atm pressure, with the CHARMM27 forcefield in NAMD, for 11 ns each. We characterized the temperature response of free energy components obtained using the MM-PBSA approach. Hyperthermophilic TBPs have a less steep response of their free energy to temperature than the mesophiles or the psycrophile, suggesting that TBP alters the curvature of its free energy surface as a global stabilization strategy; this coincides qualitatively with a measure of resiliency derived from the fluctuations of H atoms over 100 ps intervals. The van der Waals energy becomes less attractive with increasing temperature, and this correlates with a decrease in protein density and an increase in the number of sampled side-chain rotamers, but the steepness of the response is not correlated to the thermal stability of TBP. The electrostatic energy decreases upon temperature increase, and opposes all other internal energy components. This electrostatic stabilization with increasing temperature is due to the decrease in dielectric properties of water, and is reflected in an increase in the number of salt bridges at the expense of TBP-water interactions; this is particularly effective in hyperthermophiles.
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Interactions Of fluorescent Labeled Beta-amyloid Peptides With Self-assembled Nanospheres And Their Impacts On Fibrillation
Evan M. Smoak, Melanie P. Dabakis, Ipsita A. Banerjee.
Fordham University, Bronx, NY, USA.
Aggregation of amyloid β-peptide (Aβ) into fibrils plays a vital role in the pathogenesis of Alzheimer's disease. In this work, we have studied the interactions of two model peptides which include the Aβ (1-42) peptide, and the short segment Aβ (13-21) with self-assembled nanospheres. The Aβ (13-21) segment was examined as this region is believed to play a crucial role in the beta-sheet formation and fibrillation. The nanospheres were prepared by self-assembly of a newly synthesized bolaamphiphile, bis(N-α-amido-methionine)1,5 pentane dicarboxylate. We observed that the Aβ peptides showed significant binding to the nanospheres, which resulted in inhibition of beta-amyloid fibril formation. The binding of amyloid β peptides to the nanospheres was confirmed by fluorescence spectroscopy, zeta-potential analysis, FTIR, circular dichroism analysis and TEM analysis. We found that the binding of the amyloid β-peptides to the nanospheres was pH, temperature and concentration dependent as well as depended upon the hydrophobicity/ hydrophilicity of the solvent systems. Upon interacting with the nanospheres, a significant transition from β-sheet structure to alpha-helix or random coil formation is observed under acidic to neutral conditions, while relatively less interactions are observed under basic conditions. This indicates that the electrical charge density of the nanospheres play a significant role in the binding interactions with the peptides. Significant fluorescence quenching was observed when the fluorescent labeled beta-amyloid peptides were bound to the nanospheres under certain conditions. The apparent binding constants calculated were in the order of $K = 20$ to 200 M^{-1} depending on the experimental conditions. It appears that the self-assembled nanospheres may mimic the vesicles formed from certain biological bilayer membranes and suggest that the nanospheres may potentially serve as a new family of bioactive agents for inhibiting amyloidogenesis.

1696-Pos Board B540
An Investigation Of Gastric-like Aspartic Proteinase Molecular Chimeras
Charity L. Parr, Rickey Yada.
University of Guelph, Guelph, ON, Canada.
Proplasmepsin II (zPMII) represents a structurally unique member of the aspartic proteinase family, with a prosegment-enzyme interaction that has never been reported. It has been a generally accepted assertion that the prosegment in pepsin-like aspartic proteinases is critical to aspartic proteinase folding, and to investigate this further two chimeric proteins were generated, one with the pepsinogen prosegment fused to the mature region of plasmepsin II (PMII) (peppro PMII) and a second with the prosegment of PMII fused to pepsin (PMIIpropep). Both chimeras were expressed from *E. coli,* however, PMIIpropep was extremely unstable and was rapidly degraded by trypsin, suggesting protein misfolding. Since a stable enzyme could not be generated PMIIpropep was not further studied. Alternatively, peppro PMII was capable of both autoactivation and synthetic substrate cleavage. In addition, both the zymogen and mature form of the enzyme had the same predicted secondary structures, suggesting that altering the PMII prosegment did not affect this level of protein conformation although the prosegment may play a role in enzyme stability. DSC and CD measurements indicated that peppro PMII had reduced thermal stability as compared to zPMII. It is proposed that this reduction of temperature stability resulted from the loss of the ability of the prosegment in PMII to stabilize the C-terminal domain of the enzyme. The ability of PMII to fold in the presence of a completely non-homologous prosegment suggests that the prosegment in gastric-like aspartic proteinases is not always critical to enzyme folding but likely plays a role in protein stabilization.

1697-Pos Board B541
Reduced Cell Surface Stability Of Rescued Herg Trafficking Mutants
Hung D. Lam, Roxana Atanasiu, Tsukasa Okiyoneda, Herve Barriere, Gergely L. Lukacs, Alvin Shrier.
McGill University, Montreal, QC, Canada.
Long QT syndrome (LQTS) is a cardiac disorder that is characterized by a prolonged QT interval on the electrocardiogram, syncope and in severe cases sudden death. LQTS type 2 is associated with loss-of-function mutations in the human-ether-a-go-go-related gene (hERG1) that encodes the α-subunit of the cardiac delayed rectifier repolarization current, IKr. hERG mutations have been most commonly associated with defects in protein trafficking that lead to channel retention in the ER. This impaired biosynthesis can be partially rescued by low temperature and/or pharmacological chaperones. We know little about the metabolic fate of rescued mutant hERG channels and whether functional recovery may be limited by altered endocytic membrane trafficking of these channels. We undertook this study to test the hypothesis that rescued mutant hERG channels are less stable in the cell membrane and are processed differently than wt channels. Initial experiments involving immunoblotting following cyclohexamide treatment indicated that mutant hERG proteins have reduced stability compared to their wt counterpart in post-Golgi compartments. To further assess the cell surface stability and rate of internalization of hERG we conducted cell surface antibody binding assays. The results of long term binding assays confirm that mutant hERG channels are less stable at the plasma membrane than wt hERG. The results of short term binding assays suggest that this may be due in part to the accelerated internalization of mutant hERG. Using fluorescent ratio-image analysis of FITC-labeled channels revealed that mutant hERG channels are targeted preferentially to lysosomes, while wt channels are recycled. These results suggest that besides the biosynthetic processing defect, a complex alteration of peripheral membrane trafficking may contribute to the LQTS phenotypic manifestation. Furthermore, the results suggest the involvement of a post-Golgi quality control mechanism responsible for the elimination of defective hERG channels.

1698-Pos Board B542
Structural and Thermodynamic Characterization of T4 Lysozyme Mutants and the Contribution of Internal Cavities to Pressure Denaturation
Nozomi Ando[1], Buz Barstow[1], Walter A. Baase[2], Andrew Fields[2], Brian A. Matthews[2], Sol M. Gruner[1].
[1]Cornell University, Ithaca, NY, USA, [2]University of Oregon, Eugene, OR, USA.
Protein pressure denaturation cannot be explained by the liquid hydrocarbon transfer model of protein denaturation derived from thermal denaturation studies. Recent studies imply that the mechanism of pressure denaturation is the penetration of water into the protein rather than the transfer of hydrophobic residues into water. To investigate water penetration and the volume change associated with pressure denaturation, we studied the solution behavior of four T4 lysozyme mutants having different cavity volumes at low and neutral pH up to a pressure of 400 MPa using small-angle X-ray scattering and fluorescence spectroscopy. At low pH, L99A T4 lysozyme expanded from a compact, folded state to an extended but partially unfolded state. The denaturation volume change correlated positively with the total cavity volume, indicating that all of the molecule's major cavities are hydrated with pressure. As a direct

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

Abhishek Singh, Sarah Hitchcock-DeGregori.
UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ, USA.

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.

1700-Pos Board B544

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

Anupriya Singhal.
Harvard University, Cambridge, MA, USA.

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.

1701-Pos Board B545

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

Ryan Thurman, Dakshinamurthy Rajalingam, T.K.S. Kumar.
University of Arkansas, Fayetteville, AR, USA.

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

Fanrong Kong, Jiejin Chen, Jonathan A. King.
Massachusetts Institute of Technology, Cambridge, MA, USA.

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.

1703-Pos Board B547

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

Jacob W. Gauer[1], Candace Lange[2], Miguel Montes[3], Kerry Fuson[3], Kristofer Knutson[1], Jesse Murphy[1], R. Bryan Sutton[3], Greg Gillispie[2], Anne Hinderliter[1].
[1]University of Minnesota Duluth, Duluth, MN, USA, [2]Fluorescence Innovations, Bozeman, MT, USA, [3]University of Texas Medical Branch, Galveston, TX, USA.

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.