Title

Evidence against a Permanently Folded Nucleation Sequence in the Intrinsically Disordered Protein Stathmin Measured by Site-Directed Spin Labeling EPR Spectroscopy

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Authors

Foltz, Gretchen
Doupey, Tonia G
Kamel, Joseph G
et al.

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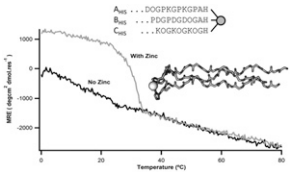
permutation on the protein chain can give important information about the connectivity, structure and folding or unfolding kinetics which guides its translocation and subsequent function in the cell. To study the process of co-translocational unfolding and its dependence on the secondary structure at the N-terminus of the protein imported, we conduct a comparative molecular dynamics study of circular permutants of Dihydrofolate reductase(DHFR) using atomistic model in CHARMM. Six Circular Permutants - CP25, CP38, CP78, CP97, CP108, CP133 - are generated such that the new N-terminus leads either to an alpha helix or a beta-strand. using Steered Molecular Dynamics we compute the work distributions for the forced unfolding of each of the CP's and native DHFR using two processes - unfolding through the geometrical constriction of the model pore as in mitochondrial translocation and mechanical unfolding with the C-terminus fixed. In both cases the unfolding force is applied at the N-terminus. A comparison of the free energy profile along the reaction coordinate for each circular permutant can lead to identification of different unfolding pathways and hence import efficiency based on first resistant structure adjacent to the targeting sequence.

2053-Pos Board B72

Design of Structral Metal Sites in Heterospecific Collagen Peptides

Avanish S. Parmar, Fei Xu, Douglas pike, Vikas Nanda.
UMDNJ, Piscataway, NJ, USA.

Globular proteins commonly use structural metals to promote folding and enhance stability. There are no good examples of similar metal utilization in fibrillar proteins such as collagen. We hypothesized a metal binding site at the end of an A:B:C-type collagen triple helix could be designed to enhance folding and stability without compromising specificity. A heterospecific metal binding site was computationally designed by sampling backbone and sidechain conformations of C-terminal amino acids of the triple helix to optimize metal site geometry. Experimental characterization of the designed sequences confirms that zinc-binding enhances structure and thermal stability of the A:B:C heterotrimer peptides under physiological buffer conditions. By varying metal concentration, it is possible to study the relative contributions of electrostatic interactions and metal binding, to triple helix stability and structure. Metal-directed switching of triple helical structure has potential applications in self-assembly of higher order biomaterials, translational regenerative medicine and drug design.



2054-Pos Board B73

Aromatic Amino Acids Confer Folding Propensities to a Nine-Residue Peptide

Olivier Bignucolo, Stephan Grzesiek, Simon Bernèche.
University of Basel, Basel, Switzerland.

We investigated how individual amino acids affect the structural propensities of short peptides. We based our work on NMR measurements of peptides of sequence EGAAXAASS, where 15 different amino acids were tested at position X. Here we focus on the two peptides with X = Trp and Gly, especially on their residual dipolar couplings (RDC). The pattern of the peptide with X = Gly was rather flat, suggesting an extended or unfolded peptide, while the pattern of the peptide with X = Trp was particularly contrasted, characterized by a changing sign value in the middle of the chain, suggesting for us the formation of a helical turn. The molecular dynamics (MD) simulations confirmed theses hypotheses. In the simulations, the peptide with X = Gly was extended most of the time and calculated RDCs were in good agreement with the experimental one. In contrast, the peptide with X = Trp showed many different conformations, mostly folded but with a non negligible number of extended conformations. After clustering the conformations according to the dihedral angles of the main chain, we found that the clusters with theoretical RDCs that better fit the experimental data were those forming a helical turn. We also show that the driving force leading to such folded conformation could arise from the lack of hydration of the peptide chain on either side of the bulky aromatic residue.

2055-Pos Board B74

Multicanonical Molecular Dynamics Simulations of Low-Molecular-Weight Peptide Oligomers

Alan van Giessen, Christine Ferry, Andrew Baird, Matthew Church.
Hobart and William Smith Colleges, Geneva, NY, USA.

We present simulation results for small peptide oligomers (dimers through hexamers) using the multicanonical simulation algorithms Replica Exchange Statistical Temperature Molecular Dynamics. This algorithms use biased dynamics to overcome sampling problems related to broken ergodicity at low temperatures. Changes in the Potential Energy Surface of the system as it grows from a single molecule to a small oligomer are discussed. Each peptide is represented using a coarse-grained model and has an alpha-helix as its native state. We show for dimers that, at low temperatures, both peptides are helical; at high temperatures, both peptides are random coils; and at intermediate temperatures, one peptide is folded while the other adopts an extended configuration that minimizes the solvent exposed surface area. Dimerization causes one peptide to become more stable and fold at a higher temperature than an isolated monomer while the other peptide becomes less stable and folds at a lower temperature than an isolated monomer. This effect is due to the solvation of the stabilized molecule by the unfolded, destabilized molecule. We show that this effect is stronger in larger oligomers and results in multiple solvated peptides that are substantially more stable than the isolated monomer at the expense of multiple molecules that are thermally destabilized. The consequences of this effect are discussed in the context of molecular crowding and of protein misfolding and aggregation.

2056-Pos Board B75

Low Resolution Structures of Cold, Warm, and Chemically Denatured Cytochrome-C via SAXS

Eric Landahl, Christopher Asta, Anthony Banks, Margaret Elmer, Trevor GrandPre.
DePaul University, Chicago, IL, USA.

The results of a small-angle x-ray scattering (SAXS) study of equine cytochrome-c protein under different unfolding conditions are discussed. Although the measured radius of gyration of this protein over a wide range of temperatures and GuHCl concentrations conform to a two-state thermodynamic model, we find different levels of residual structure present depending on whether the protein is cold- or warm- denatured. We present DAMMIN reconstructions of these different unfolded states using 1532 dummy atoms with a 1.5 Angstrom radius, and suggest ways that these different unfolded states may be described by the same folding free energy.

2057-Pos Board B76

Evidence against a Permanently Folded Nucleation Sequence in the Intrinsically Disordered Protein Stathmin Measured by Site-Directed Spin Labeling EPR Spectroscopy

Gretchen Foltz, Tonia G. Doupey, Joseph G. Kamel, Michael D. Bridges.
California State University Fullerton, Fullerton, CA, USA.

After their relatively recent discovery and classification, it has been shown that intrinsically disordered proteins (IDPs) play essential roles in cellular regulation and signal transduction. Stathmin is a eukaryotic IDP responsible for regulating the polymerization equilibrium between the tubulin heterodimer and microtubules, and is also highly expressed in cancerous cells. Stathmin exists in a highly-ordered folded state when bound to its binding partner tubulin; however, alone in solution, as an IDP lacking those stabilizing binding interactions, stathmin is likely to explore conformational space in various equilibria between partially and completely folded states. Interestingly, it has been suggested that soluble stathmin has a short helical region (Glu55-Ala73) of persistent foldedness, and that this region might act as an α-helical 'nucleation sequence' necessary for further folding in the C-terminal direction. Alternatively, we believe that the complete length of stathmin exists in a folding-unfolding equilibrium, and here we report data supporting this hypothesis. Nitroxide spin labeled mutants of stathmin throughout the Glu55-Ala73 region - rotationally immobilized through coupling to CNBr-activated sepharose beads - were studied by electron paramagnetic resonance (EPR) spectroscopy. The continuous wave EPR spectra for all 19 spin labeled mutants unambiguously indicate that this region of stathmin exists in a dynamic equilibrium between folded (ordered) and unfolded (disordered) states, and that no such helical nucleation sequence exists. We also show that this folding equilibrium in stathmin is shifted by the common biochemical viscosity modifiers sucrose, glycerol, and Ficoll-70.

2058-Pos Board B77

Why is Taq DNA Polymerase so Stable? A Reduced Entropic Folding Penalty and the Denatured State Ensemble

Chin-Chi Liu, Vincent J. LiCata.
Louisiana State University, Baton Rouge, LA, USA.

The thermal stability of Taq DNA polymerase is well known, and is the basis for its use in PCR. A comparative thermodynamic characterization of the large fragment domains of Taq (Klentaq) and E. coli (Klenow) DNA polymerases reveals that Klentaq's extreme free energy of folding originates from a significantly decreased entropic folding penalty. A parallel solution-based structural analysis reveals that the denatured state of the mesophilic polymerase (Klenow) is significantly more elongated than the denatured state of the thermophilic Taq