Posters

Protein Conformation II

1203-Pos

Molecular Dynamics Simulations Predict A pH-Dependent Conformational Change in the C-Helix of Cell Cycle Checkpoint Kinase Wee1

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Wee1 is the cell cycle checkpoint kinase that phosphorylates Tyr15 of Cdk1, maintaining it in an inactive state. Cdk1 regulates the transition from S to G2/M phases in eukaryotic cells and is activated upon dephosphorylation of residues Thr14 and Tyr15 and cyclin B1 binding. We previously reported that increased intracellular pH (pHi) (> 7.2) promotes G2/M transition. Moreover, we found that in cells having low pHi (< 7.2) the abundance of phosphorylated Cdk1 (pTyr15) is aberrantly sustained. We are testing the hypothesis that Wee1 kinase activity might be pH sensitive, with higher activity at lower pH. The C-helix in Wee1 contains four contiguous residues unique to the Wee1 family centered around His350, which faces the ATP binding site. Based on this observation, we hypothesized that changes in pHi could affect the C-helix conformation and hence catalytic activity (C-helix conformation modulates activity in many other kinases). Molecular dynamics simulations of Wee1 were performed while allowing His350 to be either neutral or charged, capturing the hypothetical protonation state of His350 at high and low pHi, respectively. Most portions of the kinase domain showed only small changes at the end of the two 10 ns simulations. However, when His350 was neutral, the C-helix adopted a conformation that closely mimics the inactive state of other kinases. In contrast, when His350 was charged, the C-helix adopted a conformation similar to the active state of other kinases wherein the C-helix was rotated towards the active site. These data suggest that pH-sensitivity in Wee1 may be mediated by the influence of the protonation state of His350 on the C-helix conformation. We are currently testing these predictions using NMR and biochemical approaches.

1204-Pos

The L49F Mutation in Erythroid Alpha Spectrin Induces Local Disorder in the Tetramer Association Region: Fluorescence And Molecular Dynamics Studies of Free and Bound Alpha Spectrin

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The bundling of the N-terminal, partial domain helix (Helix C') of human erythroid α-spectrin (αI) with the C-terminal, partial domain helices (Helices A' and B') of erythroid β-spectrin (βI) to give a pseudo spectrin structural domain (triple helical bundle A'B'C') has long been recognized as a crucial step in forming functional spectrin tetramers in erythrocytes. We have used fluorescence studies to obtain apparent polarity and Stern-Volmer quenching constants of Helix C' of αI bound to Helices A' and B' of βI. These properties were used to guide us in homology modeling with a previous NMR structure as the template. The homology models then became input structures for molecular dynamics simulations for both wild type (WT) and an αI clinical mutant Spectrin Lyon (L49F). The simulation output structures show a stable helical bundle for WT, but not for L49F. In WT A'B'C', four critical interactions were identified: two hydrophobic clusters and two salt bridges. However, in L49F, the C-terminal region of Helix C' was unable to assume a helical conformation and one critical hydrophobic cluster was disrupted. Other molecular interactions critical to the WT helical bundle were also weakened in L49F. We suggest that these conformational changes lead to a lower tetramer levels observed in Spectrin Lyon patients.

1205-Pos

Characterization of Conformational Transitions in Src Kinase using the String Method with Swarms-of-Trajectories and Markovian Milestoning

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Src tyrosine kinases are a family of signaling proteins playing key roles in intracellular signaling pathways. Dysregulation of Src activity has been implicated in several types of cancer. An important conformational change in Src involves the highly conserved DFG motif (Asp404, Phe405, Gly406) adjacent to kinase ATP-binding site. In the majority of available crystal structures, the DFG points into the ATP-binding site where it coordinates a magnesium ion (DFG-in conformation). A recent X-ray structure shows that c-Src is also able to adopt a DFG-out conformation, in which the DFG motif is flipped by approximately 180°. Understanding the factors controlling the flipping of the DFG motif is important for designing highly selective kinase inhibitors. We first determine atomistic pathway of DFG in-to-out transition using the string method with swarms-of-trajectories with all-atom molecular dynamics (MD) simulations. Images along the pathway are then used as centroids to construct a complete Voronoy tessellation in the collective variables space, which then serve as a ''basis set'' to define the discrete micro-states and to build a reduced stochastic model. MD trajectories with ''soft-wall'' restraining hyper-plane potentials are launched to keep a system inside each Voronoy cell. By monitoring the wall-to-wall transitions among the walls separating the Voronoy cells, we estimate the free energy and mean first passage time (MFPT) of DFG flipping. An improved understanding of the interplay of the structure, dynamics and activity of Src kinase will help the development of new inhibitors for targeted cancer therapy. [Supported by NIH grant CA093577]

1206-Pos

Probing the Structure of Membrane Proteins Using Pulsed EPR ESEEM Spectroscopy

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Current methods used for determining the secondary structure of membrane proteins require large sample sizes and have long data acquisition times. Therefore using pulsed EPR spectroscopic technique of Electron Spin Echo Envelope Modulation (ESEEM) is advantageous due to the requirement of less sample, short data acquisition, and high sensitivity. Using this technique, we can determine short to medium range distances (up to 8 Å) between a site-specific nitroxide spin label (MTSL) and a nearby NMR-active isotopic labeled residue for a variety of peptides and proteins which ultimately determine the difference between an α-helical and β-sheet secondary structure. The information can be obtained using a three-pulse ESEEM sequence which allows for the detection of the fundamental nuclear spin transitions. It is possible to calculate the radial distance between a paramagnetic electron and a weakly coupled nucleus because the modulation depth produced by a weakly dipolar-coupled nucleus is inversely proportional to the radial distance. This new method is applied to two different membrane peptides, M2δ AChR, and KIGAKI, which have α-helical and β-sheet structural components respectively. A nitroxide spin probe is attached to a specific Cys residue at a given position *I,* and a ^{2}H isotopic labeled residue is attached to a nearby residue ($i + 3$). The corresponding data shows that an α-helical structure will yield a very large ^{2}H peak, whereas a β-sheet will yield a much smaller ^{2}H peak, thus a difference can be observed between the two secondary structures. The ESEEM technique requires less protein sample (about 75 μg) and data acquisition only takes about 1 hour which makes it a simple, powerful, and effective technique in the determination of the secondary structure of a membrane protein.

1207-Pos

SDSL-EPR Study of a C-terminal Segment of Myelin Basic Protein in a Myelin Mimetic Environment

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Myelin basic protein (MBP) is a peripheral membrane protein and a major component of human central nervous system myelin. It is a multifunctional, highly positively charged, intrinsically disordered protein that undergoes extensive post-translational modifications. Its multifunctionality includes among others, binding of cytoskeletal proteins to a membrane surface and polymerization and bundling of cytoskeletal proteins. These interactions are modulated by interaction of MBP with Ca^{2+}-calmodulin. We report here the use of site-directed spin labeling and continuous wave power saturation electron paramagnetic resonance spectroscopy (SDSL-EPR) to examine the conformation of segment A141-L154 of a recombinant 18.5 kDa murine isoform of MBP in a reconstituted membrane environment. This segment overlaps the primary calmodulin binding region (T147-D158) of MBP and is predicted, on the basis of helical wheel constructs, to form an amphipathic alpha helix. Solution NMR investigations also showed this region to exist as a transient alpha helix in 30% TFE. Our measurements using SDSL-EPR reveal that this region forms an extended helix with a period of ~3.8 residues per turn and with a tilt angle of ~4.3 degrees with respect to the plane of the lipid bilayer. The N-terminal end of the helix appears to be buried more deeply in the membrane bilayer than the C-terminal end. The C-terminal region of the segment bears two Lys residues whose side chains interact with the lipid head groups causing this end to be more exposed. The accessibility of the C-terminal region of A141-L154, which forms a part of the primary calmodulin binding segment, could facilitate interaction of this region