

several disease-causing mutations in CIC-5 translate into truncations of the carboxy terminus (Ct), highlighting the significance of this region. The Ct interacts with the scaffold protein NHERF2 (Na^+/H^+ exchanger regulatory factor 2) and leads to enhanced endocytic uptake of albumin by kidney cells [Hryciw, D. *et al.* 2006]. This interaction occurs through the second PDZ domain of NHERF2 and an unknown binding site on the Ct of CIC-5 [Hryciw, D. *et al.* 2006]. In the present study NHERF2/CIC-5 Ct interactions were confirmed and a putative internal PDZ binding motif TSII (residues 657-60) was identified in the Ct. Mapping of this motif on the crystal structure of CIC-5 reveals it lies up-stream of a β -turn, a secondary structure element thought to be critical for PDZ domain recognition of an internal motif [Hillier, B. *et al.* 1999]. The strategic S658A mutation completely abolishes NHERF2 binding *in vitro* suggesting the PDZ binding motif was targeted. CD analysis confirmed similar spectra between wildtype and S658A mutant, indicating that this mutation does not lead to gross misfolding of the Ct. The disease mutant R648X which removes this critical S658 abolishes NHERF2 interactions providing further evidence the internal PDZ binding motif was targeted. Future work will focus on identifying proteins interacting with the CIC-5/NHERF2 complex and deciphering the role of this complex in renal endocytosis. This work was supported by the Kidney Foundation of Canada operating grant to C.B. and a NSERC studentship to L.W.

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Structural and Functional Role of Proline Residues in Fibroblast Growth Factor-1

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Fibroblast Growth Factors (FGFs) belong to a large family of β -trefoil polypeptide growth factors and contain no disulfide binds. FGFs are involved in various important biological processes such as development and maintenance of cells. FGFs are about 154 amino acids long and are believed to share a common core of 140 amino acids with 6 invariant proline residues. The secondary structural elements in FGFs include 12 antiparallel β -sheets arranged in to a β -barrel motif. Critical analyses of the three-dimensional structure of human acidic fibroblast factor (hFGF-1) show that proline residues play a critical role in shaping the β -barrel architecture of the protein. In this context, using a site-directed mutagenesis approach, we examined the effects of substitution of proline on the structure, stability and function of hFGF-1. The stability of the various proline mutants of FGF-1, were assessed by differential scanning calorimetry, limited proteolytic digestion, and urea-induced equilibrium unfolding. Conformational changes induced in the various proline mutants of hFGF-1 have been studied using 1-anilino-8-naphthalene sulfonate binding, far UV circular dichroism, and multidimensional NMR spectroscopy. The heparin and receptor binding affinities of the hFGF-1 mutants were investigated by isothermal titration calorimetry. The results of this study are expected to provide valuable information for a rational design of hFGF-1 with increased stability and enhanced cell proliferation activity.

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Structural Changes in Monomeric HIV-RT Upon Binding the NNRTI Efavirenz

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HIV-1 reverse transcriptase is a complex multi-subunit enzyme that converts single stranded viral RNA into double stranded proviral DNA. This enzyme possesses both DNA polymerase and RNase H activity. Structurally, RT is composed of a 66 kD (p66) subunit and a 51 kD (p51) subunit that lacks an RNase H domain. The homodimers retain DNA polymerase activity but only the p66/p66 homodimer has RNase H activity. In solution, the enzyme exists as an equilibrium mixture of the heterodimer p66/p51, two homodimers p66/p66 and p51/p51 and the two monomers. The nonnucleoside reversible inhibitor (NNRTI) efavirenz enhances dimerization of p66/p51, p66/p66, and p51/p51. The drug binds both monomeric and dimeric RTs. However, structural changes induced by drug binding are unknown. A combination of hydrogen deuterium exchange and mass spectrometry is used to probe the solution structure of RT monomers in the presence and absence of efavirenz. Here we demonstrate that p66 and p51 monomers have similar solution structures, which resemble the p51 subunit in the crystal structure of p66/p51 heterodimer. After drug binding the solution structure of the monomers changes to resemble the p66 subunit in the crystal structure of the heterodimer. We also show that residues in the drug binding pocket of p66/p51-efavirenz complex are affected by drug binding to monomer.

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Stabilizing Interactions In TNF Ligand-receptor Binding

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High-affinity binding of extracellular protein ligands belonging to the tumor necrosis factor (TNF) superfamily to their conjugate transmembrane cell surface TNF receptors initiates the extrinsic signaling cascade that results in apoptotic cell death. Intriguingly, despite a high-degree of structural homology among the family of ligands and receptors, the ligand binding is both high-affinity and has high-specificity. As yet, it is poorly understood what confers this specificity. Starting from the crystal structures for the TRAIL-death receptor 5 and LT α -TNF receptor 1 complexes, we used all-atom molecular dynamics simulations to investigate the stabilizing interactions between these ligand-receptor pairs. Additionally, we simulated both complexes with destabilizing point mutations. The simulation results yield insight into published experimental data as well as the underlying mechanism of high affinity ligand-receptor binding.

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Understanding the Mechanism of Autoregulation of FGF Signaling

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Fibroblast growth factors (FGFs) are heparin binding proteins that help regulate key cellular processes such as wound healing and differentiation, cell proliferation, cell migration, morphogenesis, and angiogenesis. The FGF signaling is generated by the binding of the ligand (FGF) to the extracellular domain of the FGFR, this binding induces dimerization of FGFR, which is an essential step in FGF signaling. Fibroblast growth factor receptor (FGFR) extracellular domain consists of three Ig domains D1, D2, and D3. Between the Domains D1 and D2 is a short span of acidic residues called the "acid box". The D1-D2 linker is thought to play a role in regulation of FGF interaction with FGFR. Many of the FGF binding sites can be found on the extracellular D2 domain of the receptor. It is believed that "acid box" can regulate FGF binding to FGFR. The "acid box" can mimic heparin like compounds and bind at the heparin binding sites located on the surface of the D2 region of FGFR. In the present study, we synthesized a twenty-eight amino acid box region peptide and studied its interaction with D2 domain of FGFR using various biophysical techniques including multidimensional NMR spectroscopy. Equilibrium unfolding experiment monitored steady state fluorescence, far-UV circular dichroism and proteolytic digestion experiments reveal that acid box binds to D2 domain very weakly. Two-dimensional nuclear magnetic resonance ^1H - ^{15}N HSQC experiments show that the acid box binds to the FGF-1 and heparin binding sites in the N-terminal end of the D2 domain of FGFR. Our results clearly show that the acid box peptide binds to the ligand binding domain of the fibroblast growth factor receptor.

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Interactions Defined between S100A13 and Annexin Peptides: Insight into Non-Classical Secretion

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S100A13 is a calcium binding chaperone protein, known to be involved in the non-classical export of signal peptide less proteins such as fibroblast growth factor (FGF-1) and interleukin-1 α across the cell membrane. It has also been shown that the interaction of S100A13 with Annexin II, which exhibits an inducible flip-flop mechanism across the cell bilayer, helps the multiprotein release complex to traverse the membrane bilayer. The interaction of S100A13 and annexin II has been characterized using various biophysical techniques including multidimensional NMR spectroscopy. Results of the Isothermal titration calorimetry (ITC) experiments show that holo-S100A13 exhibits preferential binding to annexin II with high affinity in the micro molar range compared to apo-S100A13. Equilibrium guanidine hydrochloride denaturation monitored by steady-state fluorescence and limited trypsin digestion analysis reveals holo-S100A13 is significantly stabilized upon binding to annexin II peptide compared to apo-S100A13. ANS (8-anilino-1-naphthalene sulfonate) binding experiments indicates that the presence of annexin II peptide does not increase the solvent availability of hydrophobic residues in holo-S100A13. Availability of the solvent-exposed hydrophobic surface(s) in apo-S100A13 does not facilitate its interaction with the annexin II peptide, which is unique characteristic of S100A13. ^1H - ^{15}N -HSQC NMR experiments reveal that the binding site of annexin II peptide on holo-S100A13 is modestly different from other S100/Annexin interactions. S100 proteins typically interact with more than one Annexin protein. In order to define the specificity of S100A13 for Annexin 2, the interaction of S100A13 with other Annexin peptides was characterized and data