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Opposing Effects of Proline and Calcium Binding Lead to Plasticity in Adhesive Dimers of Neural-Cadheirn

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Dimerization of neural-cadherins occurs via formation of calcium dependent strand-crossover structures, which leads to cell-cell adhesion in multicellular organisms. Strand-crossover dimer forms exclusively between the first N-terminal extracellular modules (EC1) of adhesive partners via swapping of their βA-sheets and docking of tryptophan-2 in the hydrophobic pocket. Prolines recurrently occur in proteins that form strand-crossover dimer and are believed to be the source of the strain in the monomer. N-cadherin has two proline residues in the βA-sheet. Our studies address two interesting questions; why is the dimerization in neural-cadherin calcium dependent, and do all three calcium-binding sites at the EC1-EC2 interface play a role in dimerization. To investigate these questions we mutated three important calcium-binding amino acids, D134, D136, and D103, and three amino acids in the βA-sheet W2, P5 and P6, in NCAD12, a construct containing EC1 and EC2. Spectroscopic and chromatographic experiments showed that the calcium-binding sites are occupied sequentially in the order of site3, then site2 and site1, and cooperativity between site2 and site1 is essential for dimerization. Studies on the P5A, P6A double mutant showed that the proline mutations increased the dimerization affinity by ~ 20 fold and relieved the requirement for calcium in dimerization. Studies on W2A showed that the binding of calcium creates strain in the hydrophobic interaction between the hydrophobic pocket and W2 in the closed monomer, which is relieved upon formation of the strand-crossover dimer. In summary, our findings confirm that the hydrophobic interaction involving W2 is the source of calcium-dependent dimerization and the proline residues at βA-sheet act as a switch to control the dynamics of the equilibrium between monomer and dimer which is crucial for the plasticity of synapses.

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Alternative Splicing at CBD2 Domain Modifies Ca2 + Sensing Properties at CBD1 in Regulatory Two-Domain Tandem of NCX Proteins

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The Na^+/Ca^{2+} exchanger isoforms (NCX1-3) and their splice variants are expressed in a tissue-specific manner and extrude Ca^{2+} from the cell to regulate Ca^{2+}-dependent events in many cell-types. The cytosolic f-loop of NCX proteins consists of two regulatory domains, CBD1 and CBD2, which form a two-domain tandem (CBD12) with a short linker. The CBD1 domain contains two high-affinity Ca^{2+} sites (Ca3-Ca4), which largely determine Ca^{2+}-dependent allosteric activation of NCX, whereas the alternative splicing sequence is exclusively located on CBD2. Previous structural, biochemical and mutational studies have shown that in an isolated CBD12 tandem, CBD2 interacts with CBD1 through a network of interdomain salt-bridges to modify Ca^{2+} affinity and off-rates at high affinity Ca^{2+} sensor of CBD1. We posit here that splice variations of A-F exons on CBD2 specifically modify dynamic properties of Ca^{2+} sensing at CBD1. In this respect, we analyzed three splice variants of an isolated two-domain construct, NCX1-CBD12: kidney (BD), brain (AD) and cardiac (ACDEF). In addition, we have analyzed CBDs from CALX-CBD12, a *Drosophila* NCX protein which is inactivated, rather than activated by Ca^{2+} binding to the high affinity sensor on CBD1. By using equilibrium binding, stopped-flow and SAXS (small-angle X-ray scattering), we demonstrate that different splice variants of CBD2 specifically modify the kinetic and equilibrium properties of Ca^{2+} sensing at CBD1. Moreover, Ca^{2+} binding decreases maximum distance (D_{max}) of the NCX-CBD12 kidney, brain and cardiac splice variants, whereas Ca^{2+} has the opposite effect on CALX-CBD12. Therefore, experimental approaches described here may help in identifying specific structural motifs in CBD2, responsible for the dynamic properties of Ca^{2+} sensing and also the mode of allosteric signal decoding.

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Dissecting the Roles of the Extracellular Regions in Apelin Peptide-Receptor Binding

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The apelin receptor is a class A G-protein coupled receptor with homology to the angiotensin II receptor and the CXCR4 and CCR5 chemokine receptors. It is expressed at high levels in lung, heart, adipose tissue, kidney, spleen and in some areas of the brain. The apelin receptor gene is also upregulated and overexpressed in one-third of human tumours. The receptor is assumed to consist of a transmembrane region with 7 helical segments, an intracellular and an extracellular domain. The extracellular domain, made up of the N-terminal tail and three extracellular loops (EL1, 2 and 3), exhibits low sequence homology to other GPCRs. Several somatic mutations in loops have been linked to diseases, pointing out the importance of loops in the activation and/or binding of GPCRs to their cognate ligands. We carried out a physicochemical characterization of the N-terminus and extracellular loops of the apelin receptor in lipid mimetic environments. The N-terminus (attached to the first transmembrane domain) and EL2 were produced as recombinant proteins while the EL peptides and a fluorescently tagged apelin were synthesized by solid-phase peptide synthesis. All species were purified by high-performance liquid chromatography. Characterization both of the peptides in isolation and of the binding between apelin and extracellular loop peptides was carried out using circular dichroism spectroscopy, fluorescence resonance energy transfer and nuclear magnetic resonance spectroscopy. These results provide insight into understanding the role of the each of the extracellular regions of this receptor at the molecular level and constitute important information the apelin receptor for the development of therapeutics targeting this system.

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High-Affinity Binding of *Aedes Aegypti* Membrane-Bound Alkaline Phosphatase to the *Bacillus Thuringiensis* Cry4Ba Toxin: Structural Implications for Toxin-Receptor Interactions

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Membrane-bound alkaline phosphatase of midgut epithelial cells from *Aedes aegypti* larvae (Aa-mALP) was previously identified as *Bacillus thuringiensis* Cry4Ba toxin receptor. Here, the 54-kDa His-tag fused ALP was overexpressed as inclusion body in *Escherichia coli* BL21. After solubilization in 8M urea, this ALP protein was refolded and purified using Ni-NTA affinity column. The refolded ALP was able to retain phosphatase activity and its binding to the activated Cry4Ba toxin under nondenaturing (dot blot) conditions. Quantitative binding analysis using quartz crystal microbalance (QCM) revealed that the immobilized ALP on gold electrode was bound by the Cry4Ba toxin with high binding affinity (*K*d ~ 14 nM). A homology-based ALP structure implies that although the overall structure is highly similar to known ALP structures from other organisms, its ligand binding properties could be different. Altogether, the data present here supports our previous notion that the binding to its counterpart toxin-Cry4Ba toxin does not rely on glycosylation of this alkaline phosphatase.

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Analysis of First and Second Shell Interactions in Phosphate-Binding Proteins

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The phosphate anion is involved a wide range of processes ranging from cell signaling to energy storage in cells[1]. It can interact with proteins in different modes, where its interactions range from being covalently bound to the protein to coordinating metal sites in enzymes[2]. The motif for coordinating or binding the phosphate depends on its functional usage and e.g., in ATP or GTP binding proteins a structural motif named the P loop is often found[3]. Only few of these structural motifs have been studied in great detail where quantum mechanical computations will give an elaborate description of the interactions. In this work, we survey phosphate-binding proteins with emphasis on the molecular recognition of the first- and second-shell interactions between anion and amino acid residues. To characterize the binding sites, we optimize the geometries by using density functional theory calculations. From the optimized geometries, we calculate the charge transfer and force constants between the first shell interactions and the phosphate moiety as well as the interaction between the first- and second shell of the protein. The results describe the strength of the first shell interaction with the anion and the importance of the second shell to support the binding motif. This knowledge is of importance in understanding phosphate binding proteins and in the development of biomimetic sustainable phosphate biosensors.

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