Computational Prediction and Experimental Validation of a Novel Binding Site for Platelet Integrin AlphaIIb-Beta3

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simulation which is the most time demanding step. In this work we demonstrated a possibility to reduce time of conformational sampling using crystal environment simulation. Amber10 program and FF99SB/GLYCAM06 force fields combination were employed for MD simulations and free energy calculations. Six 3, 4 and 3,4-deoxy dimannose analogs were studied as ligands of the m4-P51G-CVN mutant of the potent anti-HIV carbohydrate binding agent Cyanovirin-N (1). The use of crystal over solution simulations results in at least 8 times faster generation of the equivalent length trajectories. Binding free energy ΔA estimated from crystal NVT simulation trajectories shows 0.93 and 0.94 correlation with ΔG from solution NPT simulations for MM/PBSA and MM/GBSA approaches, respectively. We also evaluated performance of the relatively new GLYCAM06 carbohydrate force field and found reasonable agreement between calculated ΔG and experimental value. Results of this study further support our earlier hypothesis about importance (for CVN specificity) of the eight-component H-bond interactions of dimannose and protein main chain atoms that also was recently observed in experiment. [1] Vorontsov and Miyashita (2009) Biophys. J., 97.

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Computational Prediction and Experimental Validation of a Novel Binding Site for Platelet Integrin AlphaIIb-Beta3

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Fibrinogen mimetic drugs and a γ-chain peptide bind to both the αIIb and β3 subunits of integrin αIIbβ3, providing the final coordination site for the metal ion-dependent adhesion site (MIDAS) metal ion within the β3 I domain, and inducing a less compact conformation of the protein. We investigated the binding of a novel human-selective αIIbβ3 small molecular inhibitor (RUC-1) that we recently identified by high throughput screening, and predicted to bind preferentially to a pocket within the αIIb β-propeller domain by flexible ligand/rigid protein molecular docking studies. We first carried out both standard and enhanced molecular dynamics (MD) simulations of the proposed RUC-1-bound form of human αIIbβ3 integrin. The results of these studies pointed to an energetically preferred conformation of RUC-1 into the αIIb β-propeller domain that had no contact with the MIDAS metal ion or other sites in the β3 I domain. This binding mode of RUC-1 appeared to be stabilized by interactions with specific human αIIb residues, such as D224 (already known to contribute to αIIbβ3 binding), Y190 (F in both mouse and rat), and (through two water molecules) D232 (H in rat). Well-tempered metadynamics simulations of Y190F and D232H mutants supported the contribution of normal residues to the stabilization of RUC-1 in a specific binding mode and location. Functional experiments on recombinant cell lines expressing Y190FαIIbβ3 or D232HαIIbβ3 validated this hypothesis by showing a ~80-95% reduction in RUC-1 affinity. X-ray crystallography confirmed the RUC-1 binding pose suggested by MD simulations, while gel filtration and dynamic light scattering experiments showed that RUC-1 favored a compact αIIbβ3 conformation, in sharp contrast to the effect of fibrinogen-mimetic drugs.

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Membrane Binding and Lipid Extraction Studies of Gm2 Activator Protein (GM2AP)

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GM2AP is an accessory protein that solubilizes the ganglioside GM2 from intralysosomal vesicles for hydrolytic cleavage by HexA to form GM3. The precise molecular interactions and method of extraction of GM2 from lipid vesicles are unknown. GM2AP also functions as a lipid transfer protein. This non-enzymatic protein contains three tryptophan residues (W5, W63, W131) with two of these (W63, W131) located in putative membrane binding loops. In order to investigate the possible role of the tryptophan (TRP) residues in membrane binding and lipid extraction, gel filtration and resorcinol absorption assays were used to investigate the extraction efficiency of GM2 by GM2AP in a series of TRP to ALA substituted constructs. GM2AP is shown to have two distinct substrate binding modes, one for the GM2 ganglioside and another for phospholipids. Fluorescence experiments were used to determine the orientation of dansyl-DHPE in the binding pocket of GM2AP. Quenching results suggest that dansyl-DHPE is oriented such that the head group of the lipid is located in the hydrophobic pocket of the protein, consistent with the binding mode of other phospholipids which were previously studied. Dansyl-labeled lipids were used to monitor the changes in the rates of lipid extraction and transfer by GM2AP from liposomes as a function of both pH and the TRP to ALA substituted constructs. The ability of GM2AP to bind and/or extract dansyl-labeled lipids from liposomes was affected with increased pH of the lipid environment. Additionally, removal of TRP from the putative membrane binding loops resulted in slower lipid extraction rates, suggesting that these residues are relevant for membrane binding and/or extraction of GM2AP.

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Extracellular pH and Regulation of Integrin-Ligand Interactions

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In the tumor and wound microenvironments, the extracellular matrix often exhibits acidic extracellular pH. It is well known that acidic pH can strongly affect processes such as cell adhesion and migration. However, the molecular mechanisms governing these effects have not been established. Here, we consider the hypothesis that acidic extracellular pH directly alters the interactions between cell surface integrin receptors and ECM ligands, which are critical to cell adhesion and migration. We employed Multi-Conformation Continuum Electrostatics to predict amino acid pKa values in the integrin avb3 headpiece, and conducted molecular dynamics simulations at acidic and physiological pH to examine the effect of pH on integrin conformational states. Our results suggest that acidic pH promotes opening of the avb3 headpiece, an important step in activation that can enable more effective ligand-receptor association interactions. This has important implications for downstream cell processes in the cancer and wound environments. We also conducted molecular-level experimental approaches, including flow cytometry and atomic force microscope-enabled force spectroscopy, to further examine the role of pH in regulating integrin-ligand interactions. These molecular-level results are connected to cell-level measurements of adhesion and migration at different pH levels, providing a detailed, multi-scale understanding of how acidic extracellular pH affects cell behavior.

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Effect of Molecular Sway on the Recognition of Peptide/MHC Complex by T Cells

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CD4+ T cell responses require the recognition of specific peptide-MHC complexes displayed by APC. It is important to determine how antigen presentations affect the ensuing T cell response. Immunizations of B10.BR mice with naturally processed peptide 48-61 of Hen egg lysozyme elicit two different types of T cell responses. First type of T cell (type A termed by Unanue et. al) respond to APC pulsed with either peptide or whole HEL protein. Second type of T cell (termed type B) respond to APC incubated with peptide but showed no response to APC pulsed with whole protein. Reactivity of the type A T cell clones correlated well with the affinity of the peptide to the MHC molecules. However, some type B T cell clone exhibit better response to the low affinity truncated (52-61) peptide than to high affinity peptide (48-61). Since weak MHC binding peptides form unstable complex, we hypothesize that type B T cells respond to the transitional conformations generated by unstable peptide/MHC complex. To test this hypothesis, we analyzed the movement of peptide/MHC complex at the single molecular level by using diffracted X-ray tracking (DXT) method. It was found that movement of the low affinity peptide/MHC complexes was different from that of high affinity peptide/MHC complexes. Moreover, comparison of the movement of a series of low affinity peptide/MHC complexes revealed clear correlation between magnitude of twisting movement of peptide and T cell recognition. Thus, our results clearly demonstrated that movement of peptide in MHC strongly affects to T cell recognition and some but not all T cells recognize a transitional conformation generated by weak binding peptides.

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A Force Spectroscopy-Based Protein-Ligand Interaction Assay

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Binding of small molecules are crucial to the function and folding of many protein machineries inside cell. Thus it is of fundamental importance to measure the binding affinity of small molecule ligands to proteins and reveal the binding mechanism. Here we report a force spectroscopy based single-molecule binding assay that is capable of determining the binding affinity as well as the binding mechanism of ligands to proteins at the single-molecule level. This assay is based on the difference in the mechanical stability of the given protein upon ligand binding. As a proof-of-principle, we use the binding of metal ions, Ni2+, to an engineered metal binding protein, G6-53, as a model system to establish this method. The apo-G6-53 and Ni2+-bound G6-53 exhibit distinct mechanical stability: apo-G6-53 unfolds at around 120 pN while Ni2+-bound