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Molecular Modelling Studies of Bovine and Camel Chymosin-κ-Casein Complexes

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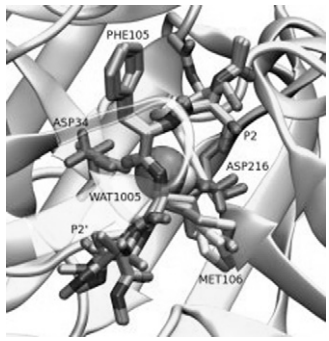
We present computational studies of two homologous mammalian aspartic proteases (calf and camel chymosin) complexed with 16-residue fragments of their native peptide ligands (cow and camel κ-casein) and the cross-complexes. Using molecular docking calculations, homology modelling and molecular dynamics simulations, we compare the binding modes of the four systems. The complexes are of industrial interest because camel chymosin has recently been marketed as an alternative to bovine chymosin as an enzyme to clot milk in cheese manufacturing. The camel enzyme has been shown to have 70% higher clotting activity and only 20% of the unspecific protease activity for bovine κ-casein as compared to the bovine enzyme. Interestingly, bovine chymosin has a very low proteolytic rate for camel κ-casein. The models provide putative atomic coordinates for these complexes, for which there are no available crystallographic or NMR structures, and help to explain some existing experimental results.



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Changes in HIV-1 Protease-Inhibitor Interaction Due to Amino Acids Polymorphisms and Drug-Pressure Selected Mutations

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Both pulsed electron paramagnetic resonance (EPR) and 2D HSQC NMR are used to study HIV-1 protease-inhibitor binding by monitoring the changes in flaps conformation and amide chemical shifts. For all NMR and EPR experiments, here, we used an inactive (D25N) enzyme construct and nine FDA approved inhibitors. Results show that several inhibitors induce a closed flap conformation and cause significant splitting of the amide backbone chemical shifts due to a removal of the homodimer symmetry upon binding. Other inhibitors are found to interact more weakly with the protein, resulting in incomplete flap closures and perturbation of HSQC peaks due to chemical exchange. Qualitatively, both the pulsed EPR and NMR methods can discern different degrees of protease-inhibitor interactions, which we define as strong, moderate, and weak. The stability of each of the protease-inhibitor formed was determined by measuring the transition midpoint (Tm) with differential scanning calorimetry (DSC). Inhibitors classified as ''strong binders'' have higher Tm values. To determine changes in protease-inhibitor interaction with drug resistant constructs, we used the inactive sub-Saharan HIV-1 protease (8 amino acid polymorphisms), MDR769 (11 mutations) and V6 (8 mutations). Our pulsed EPR and NMR results show differences in inhibitor interactions in subtype C, MDR769 and V6 compared to subtype B.

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Interactions Within the E2 Enzyme Cdc34-Ubiquitin Complex Are Transient

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The process of ubiquitylation involves the transfer of the small 76-residue protein ubiquitin (Ub) between a series of enzymes (E1, E2, E3) until it binds to a lysine residue of the substrate protein. When repeated several times, the E1-E2-E3 cascade forms a K48-linked polyubiquitin (poly-Ub) chain targeting the protein substrate for 26S proteasomal degradation. The E2 enzyme is the key protein in this cascade as it must recognize both the E1 and E3 enzymes, label the substrate, as well as form a covalent thiolester with Ub. Cdc34 is a class II E2 conjugating enzyme in the Ub-dependent degradation pathway of cell cycle proteins. It is comprised of a ~170 residue catalytic domain, common to all E2 enzymes, which contains the active site cysteine (C93). Two unique features of cdc34 are its acidic loop (residues 102-113) and acidic tail (residues 171-236), both of which are required to produce poly-Ub chains. However, the underlying mechanism that these regions have on chain assembly remains unclear. We have used NMR spectroscopy to study protein-protein interactions between cdc34 and Ub by using a stable covalent disulphide cdc34-Ub to mimic the thiolester complex. Chemical shift mapping was used to identify sites of non-covalent interactions in the cdc34-Ub to create a model of the complex. Competitive binding studies using free Ub or the cdc34 tail (residues 183-236) with cdc34-Ub indicate that transient non-covalent interactions exist between cdc34 and Ub. These studies provide the first detailed structural information to explain the unique mechanism used by cdc34 to promote poly-Ub chain assembly.

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Binding of Small-Molecule Inhibitors To MAP Kinase ERK2, Studied with Resonance Energy Transfer (RET)

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The extracellular-signal-regulated kinase (ERK) proteins belong to the mitogen-activated protein kinase family. They participate in several important cell-signaling pathways. Unregulated, ERK2 is thought to mediate cell proliferation in many types of cancer. But because ERK also functions in many normal cell activities, inhibition selectivity is necessary for its use in cancer therapy: one needs to block only phosphorylation of those substrates that are involved in cell proliferation, while leaving phosphorylation of other substrates unaffected. Novel small-molecule compounds, which inhibit phosphorylation of selected substrates but do not compete with ATP for its binding site, have been previously identified by computer-aided drug design (CADD) and molecular-dynamics docking algorithms. Herein, we spectroscopically characterized selected compounds, and show that they can serve as resonance energy transfer (RET) acceptors for tryptophan: we determined the values of spectral overlap J between the compounds' absorption and ERK fluorescence, and of the Forster distance R_0 for the compound/tryptophan pairs. We then used RET to determine average distances between the bound compounds and the group of three tryptophans in ERK2, thus validating the CADD predictions of the compounds' binding sites. The problem of multiple but closely located donors (tryptophans) that pass the energy to the single acceptor (the bound compound) is discussed, as is the possibility of utilizing RET for mapping the inhibitor-binding sites on other kinases. The novel RET acceptors for tryptophan can conceivably be employed in studies of other protein/ligand systems with suitable spectroscopic properties.

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Receptor Transactivation Measured in Live Cells Using Spatial Intensity Distribution Analysis (spida)

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Recent evidence suggests that signaling responses elicited by G-protein coupled receptors (GPCRs) are more complex than first believed. For instance, stimulation of several GPCRs leads to the transactivation of receptor tyrosine kinase (RTK) signaling pathways. Many groups have studied the role and mechanistic details of RTK-GPCR cross communication, using standard biochemical methods. While these studies have helped to elucidate possible intermediates involved in this complex network, the diversity of cell types and receptors used has often lead to conflicting results and provided little quantitative information.

We present a quantitative measurement of RTK transactivation, using spatial intensity distribution analysis (SpIDA). Employing confocal microscopy, spatial intensity histograms are fit using super-Poissonian distributions, yielding molecular parameters such as density and particle quantal brightness. SpIDA provides measurement of particle densities and oligomerization states of receptors using single images as input. Thus processes such as transactivation, which results in changes in the oligomerization state, can be monitored. CHO-K1 cells expressing EGFR-GFP were transfected with various GPCRs including β2-arrestin, neurokinin1, angeotensin and dopamine receptors. We monitored the relative distribution of monomeric and dimeric EGFR in response to GPCR stimulation and obtained dose response curves for different EGFR-GFP:GPCR interactions.

To confirm our findings, similar dose response curves were obtained using FLIM-FRET data and negative controls were also measured when activation or transactivation was blocked with a variety of biochemical agents including Rab5 and AG1478.

By fitting dose response curves to standard models, key parameters such as D50, and Dmax were obtained. To show that those results were not biased by either cell type choice or overexpression, we studied transactivation in situ in primary