Molecular basis of simvastatin binding to integrin αXβ2

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Introduction

Integrin αXβ2 (CD11c/complement receptor 4), a heterodimeric cell surface receptor, is exclusively expressed in leukocytes and functions in cellular trafficking, phagocytosis, and T-cell proliferation.

The importance of the αXβ2 is illustrated by its mutation in leukocyte adhesion deficiency, a lethal disease. Upon ligand binding, the ligand-binding domain of αXβ2, called the αX I-domain, undergoes conformational changes.

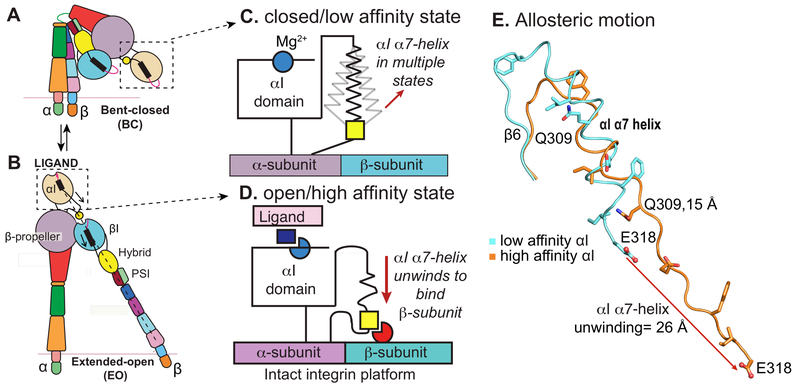


Figure 1: Conformational changes between (A,C) bent, closed/low-affinity and (B,D) extended, open/high-affinity conformations of the integrin αXβ2. (E) Schematic of the αI-α7 helix motion during integrin activation.

The αX I-domain is an allosteric protein that relays bidirectional cellular signaling between the α and the β subunits of the αXβ2 through allosteric coupling between the divalent cation binding site and its allosteric αI-α7 helix.

The binding of Mg^{2+} and subsequent extracellular ligand induces reorganization of the αX I-domain, hypothesized to move the αX I-domain from the closed to the open state and induces piston-like downward movement of the αI-α7 helix.

The carboxyl group of the hydrolyzed simvastatin, which is a small molecule, was found to antagonistically bind Mg^{2+} at the Metal Ion Dependent Adhesion Site (MIDAS) of the αM I-domain, a sister homolog of the αX I-domain.

Hypothesis:

1) Simvastatin binds to the αX I-domain.
2) Simvastatin stabilizes the open state conformation of the αX I-domain.

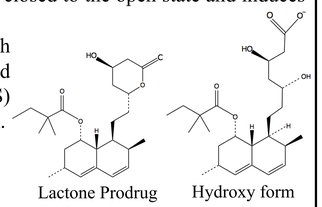


Figure 2: Chemical forms of simvastatin.

Objective

The aim of this study is to characterize the binding mode of simvastatin to the αX I-domain at the atomic details and design 2nd generation selective molecules for the αXβ2.

Simvastatin blocks ligand binding of the αXβ2

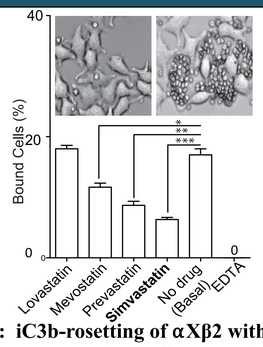


Figure 3: iC3b-rosetting of αXβ2 with statins. The strongest antagonistic effect was observed for 10 μM simvastatin on binding of the αXβ2, to its natural ligand, iC3b,by using the rosetting assay.

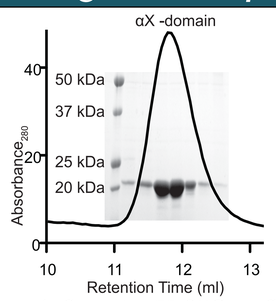


Figure 4: Superdex-75 SEC profile and SDS-PAGE of αX I-domain. The 22 kDa αX I-domain, was expressed using *E. coli* Rosetta cells and then purified by affinity and size exclusion chromatography.

Direct interaction of simvastatin to the αX I-domain

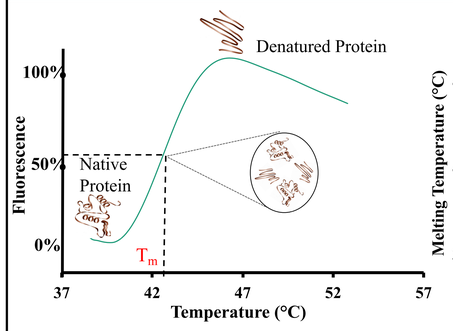


Figure 5: The thermal shift assay.

The fluorescence was measured for 11.4 μM αX I-domain containing sypro orange dye. Measurement taken at regular intervals with the temperature gradient of 0.1 ºC per 1 minute over a temperature range spanning from 15 ºC to 95 ºC.

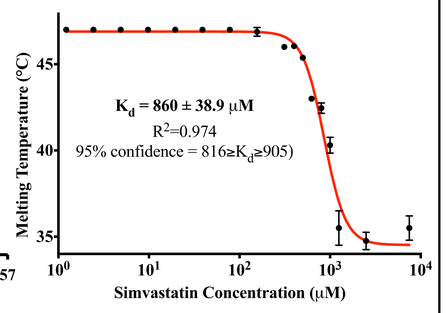


Figure 6: Determination of Simvastatin affinity for the αX I-domain using DSF.

- An increase in the concentration of simvastatin from 1–7500 μM resulted in a decrease in the melting temperature.
- Simvastatin binds to the αX I-domain.

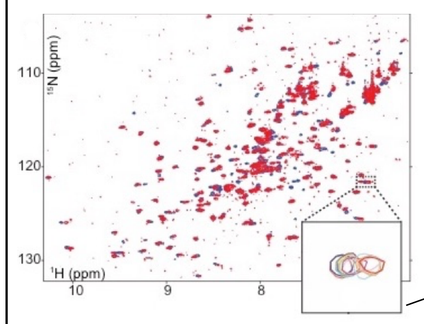


Figure 7A: Nuclear Magnetic Resonance (NMR) titration of the αX I-domain with increasing concentration of simvastatin. HSQCs of 25 μM αX I-domain in 10 mM sodium phosphate, 150 mM NaCl, 10% D_2O, pH 6.4 were acquired on Bruker 800 MHz at varying concentration of simvastatin.

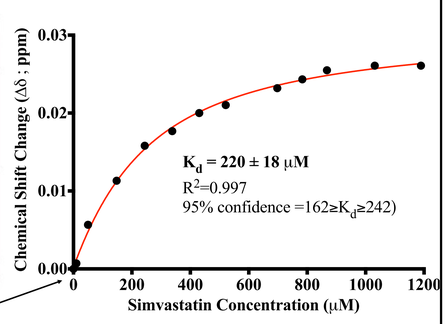


Figure 7B: Chemical shift perturbation upon simvastatin binding.

- Affinity measurement of αX I-domain to simvastatin by NMR agrees with the DSF result.
- Provides a feasible platform to perform further NMR dynamic studies.

Molecular basis of simvastatin binding

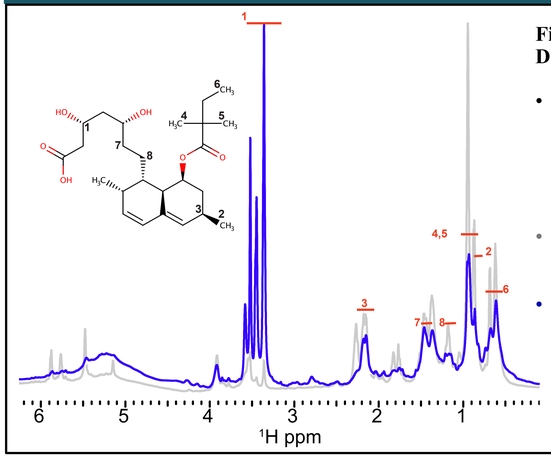
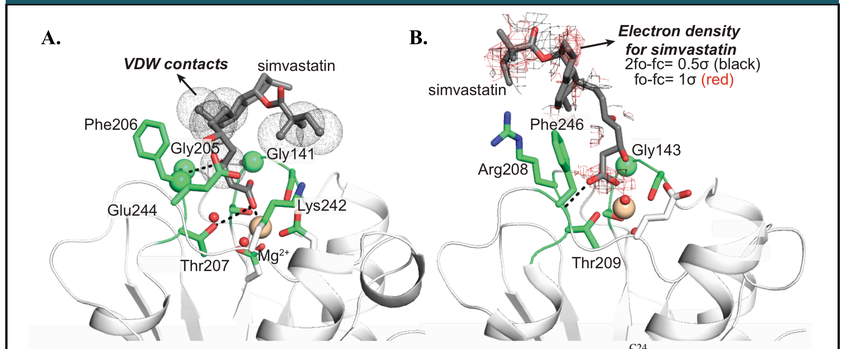


Figure 8: Saturation Transfer Difference (STD)

- NMR spectra were obtained for 25 μM αX I-domain in 20 mM sodium phosphate, 100 mM NaCl, 10% D_2O, pH 6.4 in the presence of 1 mM simvastatin.
- Grey. 1D NMR spectrum of the simvastatin.
- Blue. STD NMR spectrum revealing directly interacting residues of the αX I-domain with simvastatin based on the transfer of magnetization from the αX I-domain.

Molecular docking of simvastatin to the αX I-domain



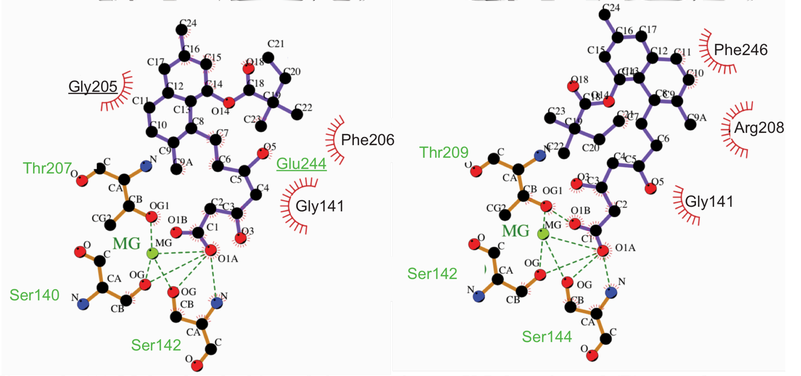


Figure 9: (A) Molecular docking of simvastatin to αX I-domain and (B) crystal structure of αM I-domain-simvastatin complex.

A) The carboxyl group of hydrolyzed simvastatin associates with Mg^{2+} at the MIDAS through hydrophilic interactions with Thr207, Ser140, Ser142, and Glu244 (shown in dotted lines), whereas the aromatic moiety of simvastatin interacts with Phe206, GLy205, and Gly141 through the van Der Waals interactions (shown in shaded spheres).

B) The negative electron density of simvastatin (shown in red wire) potentially indicates that structural assignment of simvastatin in its crystal complex with the αM I-domain might not be the best representation for demonstrating the molecular basis of simvastatin binding.

Discussion

- The decrease in the melting temperature of the αX I-domain in DSF, as well as the instability of the protein during STD NMR, were observed upon the addition of simvastatin. These observations indicate that the binding of simvastatin to the αX I-domain potentially induces conformational change. Since the carboxylate group binds to the ligand binding site of the protein, the αX I-domain perhaps changes towards the open state.
- Even though simvastatin antagonizes ligand binding affinity to the α αX I-domain, it could potentially still induce signaling on the cell surface for internalization of αXβ2.
- Despite 66% homology between the αX and the αM I-domains, the specificity in their respective ligand-binding modes differ. The insights on the molecular basis of simvastatin binding to the αX I-domain could be significant for investigating potential binders, such as small molecules or peptides, which could in turn help in designing drug compounds for inflammatory diseases.

❑ **Future Directions:** We plan to perform NMR dynamics studies and X-ray crystallography in both the presence and absence of simvastatin in order to elucidate the in-depth atomic details of simvastatin- αX I-domain interactions.

References

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Acknowledgements

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