TRIPLE RESONANCE NMR BACKBONE ASSIGNMENT OF THE LIGAND BINDING DOMAIN OF THE αxβ2 INTEGRIN

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ABSTRACT

The αxβ2 integrin is a heterodimeric cell surface receptor exclusively expressed in leukocytes and plays a vital role in monocyte adhesion and atherosclerosis development. It functions in cellular trafficking, phagocytosis, and T-cell proliferation. Importantly, drugs targeted to αxβ2 could potentially have an anti-inflammatory effect without causing global immune suppression associated with steroids. The ligand binding domain of αxβ2, called the αxI-domain, transitions from closed/low affinity to open/high affinity states upon binding to a ligand.

In order to design medications targeted to this protein and understand the mechanism of its transition between the open and closed conformational states, structural studies must be undertaken. Of particular interest are structural studies of the ligand-binding αxI-domain, which undergoes drastic conformational changes in the transition between open and closed states. While there are published crystal structures of the αxI-domain in the open and closed states, these structures fail to show how the transition between these two states occurs, and the flexibility of the domain. Nuclear Magnetic Resonance (NMR) studies can bridge this gap by allowing for probing molecular motions at the nanosecond timescale.

Hence, as a preliminary project, we have conducted an NMR backbone assignment of the αxI-domain. The αxI-domain plasmids are transformed to E. coli Rosetta cells. 15N/13C-labeled and 13N/15C-labeled samples were purified by affinity and size exclusion chromatography. Differential Scanning Fluorimetry (DSF) was used to determine the NMR buffer in which the protein was most stable. Triple resonance experiments such as HNCACB, HNCCACB, HNCO, and HNCACO were used to assign residues in the HSQC spectra.

RESULTS

Protein Purification

Figure 1: Efficiency chromatography purification of triple labeled αxI domain using a HiLoad Superdex 200 16/60 column. Protein was eluted increasing the concentration of Buffer B relative to Buffer A in a linear gradient. A) Size exclusion chromatography (Superdex 200 16/60) of triple labeled αxI domain. Both affinity and size exclusion steps were performed at 4 °C, with flow rates of 0.5 ml/min and 2 ml/min, respectively.

Figure 2: Efficiency chromatography purification of triple labeled αxI domain using a Hitrap Histrap column. Protein was eluted increasing the concentration of Buffer B relative to Buffer A in a linear gradient. B) Size exclusion chromatography (Hitrap Histrap) of triple labeled αxI domain. Both affinity and size exclusion steps were performed at 4 °C, with flow rates of 0.5 ml/min and 2 ml/min, respectively.

Figure 3:Assigned HSQC Spectra 15N/13C-labeled αxI Domain

Figure 4: Strip plots from HNCACO and HNCACB spectra acquired at 298 K with 5 mg/mL 15N/13C-labeled αxI domain in 10% D2O. Bold plots from HNCCACB and HNCACB spectra acquired at 298 K with 5 mg/mL 13N/15C-labeled αxI domain in 10% D2O. Strips colored in red indicate peak splitting.

Figure 5: Overlaid Crystal Structures with Residues Showing Peak Splitting Highlighted

CONCLUSIONS

- 15N/13C/1H and 13N/15C labeled αxI Domain expressed in E. Coli Rosetta Cells
- Isotope labeled protein purified by size exclusion and Ni-NTA chromatography
- Acquired HSQC, HNCCACB, HNCCACB, HNCO, HNCCACB, HNCCACB, HNCCACB, HNCCACB spectra
- 165/166 (87.7%) of assignable residues assigned in HSQC (not including proline)
- Peak splitting present for many residues in allosteric regions of the protein (including the α2β2-loop)
- This suggests that the protein takes on more than one ensemble of conformations in solution
- The αxI Domain may take on the open conformation in solution

REFERENCES


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