Structural Characterization of Leukocyte Integrin αM I-Domain

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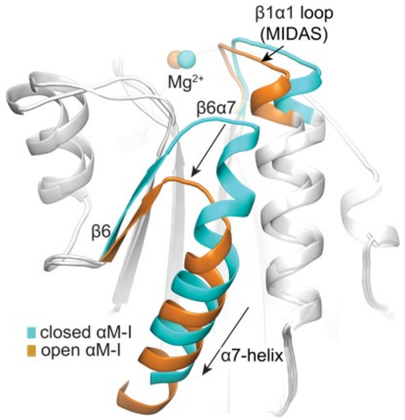
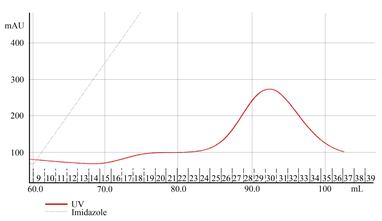
Background

The aim of this study is to characterize the stability of the ligand-binding site (also called the αI-domain) on the cellular adhesion molecule, integrin αM (ITGAM). This integrin is involved in the migration of leukocytes out of the blood stream, making it an interesting target for pharmaceutical therapies. Within this αI-domain lies a Mg^{2+} binding site called the Metal Ion Dependent Adhesion Site (MIDAS), which is allosterically linked to the domain's C-terminal helix. Recent findings on its closest sister analog have shown that activation of this receptor causes the C-terminal helix to reshape and lose much of its helicity. We investigate the stability of the αI-Domain by analyzing its melting temperature at varying concentrations of Mg^{2+}.

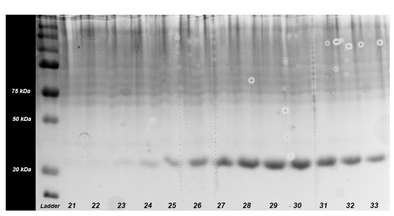


Mg^{2+} binding induces change from closed to open. Binding at the MIDAS site causes the C-terminal α7-helix to reshape from the closed confirmation (blue) and shift two turns downwards in position in the open state (orange).

Expression and Purification of αM I-Domain using His-Tag

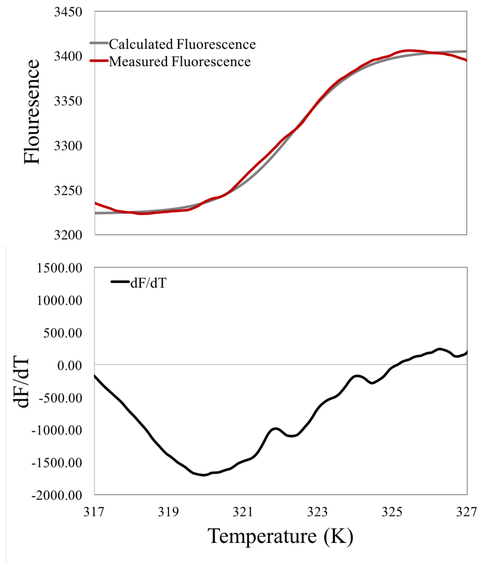


Chromatogram from 5mL Nickel-NTA Column. The protein was loaded onto the column and eluted with increasing imidazole concentration. The peak ranging from 85-105 mL shows the elution of our protein of interest.



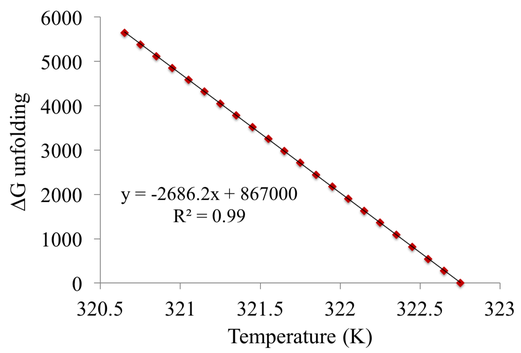
12.5 % SDS-PAGE. The gel depicts bands slightly above the 20 kDa protein marker indicating the presence of purified αM I-domain. The lane numbers correspond to the fraction number from the chromatogram.

Differential Scanning Fluorimetry



Fluorescence of SYPRO Orange vs. Temperature (K). The first plot (top) depicts the measured fluorescence of SYPRO Orange dye as it binds to the hydrophobic regions of the I-domain. The data was fitted using a Boltzmann distribution (grey). As the protein is subject to higher temperatures and begins unfolding, more hydrophobic from the interior of the protein become available for binding. This allows us to visualize the unfolding by increasing monitoring the increase in fluorescence. The second plot (bottom) depicts the first derivative of the fluorescence vs. temperature. The data shows that near 320-325 K the protein unfolds rapidly.

Determination of Thermodynamic Parameters of αM I-Domain Unfolding

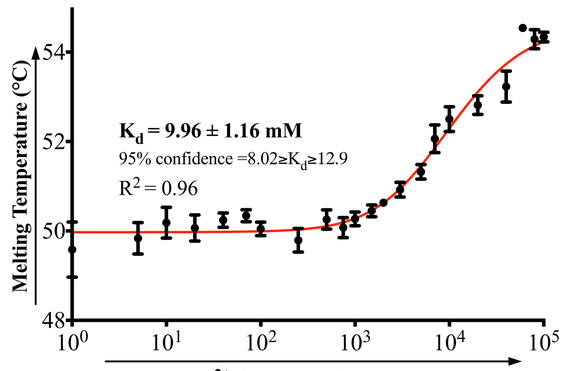


Gibb's Free Energy of Unfolding vs. Temperature (K). The linear region of the raw fluorescence data was used to create a linear plot in the form of $y = mx + b$ corresponding to the thermodynamic equation

$$\Delta G = -T\Delta S + \Delta H$$

The value of ΔG was then determined at T = 293 K to be 79931 J/mol. This high positive value means that the unfolding is highly non-spontaneous. $\Delta H$of 866853 J/mol indicates the change from folded to unfolded state is a highly endothermic reaction. These calculations were performed using the method described by Thaiesha A. Wright et al.

Mg^{2+} Increases αM I-Domain T_m



Mg^{2+} vs Melting Temperature of αM I-Domain. The points represent average melting temperature at varying concentrations of Mg^{2+}. The data was fit using non-linear regression. The points trend upwards, indicating that increasing magnesium concentration results in higher melting temperatures, potentially as a result of increased stability. Fitting the data, we estimated that the point at which the protein is fifty-percent saturated i.e. the disassociation constant (K_d) for binding of Mg^{2+} to the I-domain is 10mM.

Discussion

The data shows that increasing Mg^{2+} results in a higher melting temperature, indicating an increase in stability. However, the preliminary nature of these results calls for additional thermofluor assays to further elucidate the effect of magnesium binding on the C-terminal helix. Additionally, NMR studies would be useful to understand the structure of this domain in solution. The results of this work will allow high throughput screening that could be employed to discover possible allosteric inhibitors for macrophage integrins. Therefore, this protein is a highly suitable target for use in novel immunotherapies.

References

A. Wright, Thaiesha & M. Stewart, Jamie & Page, Richard & Konkolewicz, Dominik. (2017). Extraction of Thermodynamic Parameters of Protein Unfolding Using Parallelized Differential Scanning Fluorimetry. The Journal of Physical Chemistry Letters.

Acknowledgements

This study was supported by the University of Houston Summer Undergraduate Research Fellowship and the Provost's Undergraduate Research Scholarship programs. Special thanks to all members of the Sen Lab who shared their time and expertise to support this project.