Understanding Conformational Regulation of the Integrin I-domain for Design of Chimeric Protein Switches

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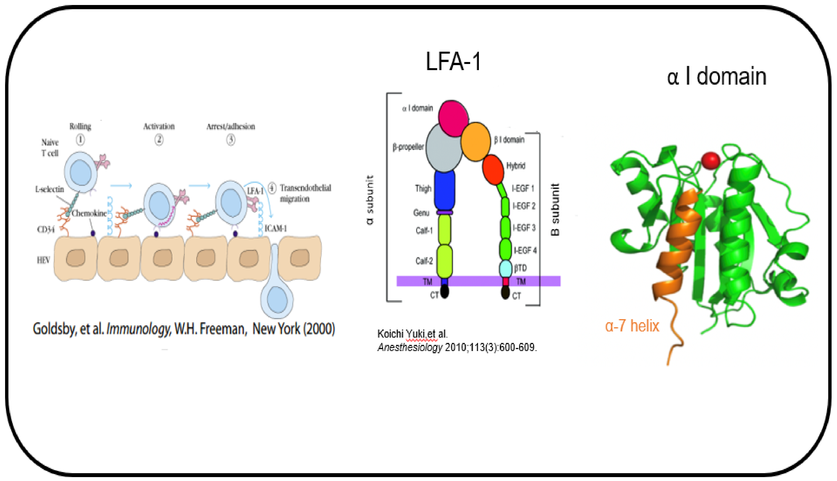
Abstract

Many proteins express multiple allosterically regulated conformational states, with protein function regulated by effector molecules, environmental responses and other ligands. One such protein is the LFA-1 surface integrin protein and its inserted domain, the I-domain. We Isolated the I-domain for investigation of determining binding properties and understanding conformational regulations of affinity changes to its target ligand ICAM-1, for further use in chimeric protein switch design. A large change in binding affinity was found through the disruption of a sub-sequence of amino acids in I-domain known as the α7 helix. When the α7 helix is deleted, I-domain converts into a *permanent high affinity state* in which binding affinity to ICAM-1 was increased. (This state can be reversed by co-expression with soluble α7 helix peptide.) These results conclude that the α7 helix stabilizes the I domain in its low affinity conformation in a ligand-like manner, allowing relaxation to the high affinity conformation upon disruption of α7 helix interaction. α7 helix deletion I-domain can not be applied in design of chimeric protein switches due to its permanent conformational state. Switch design has a focus of allosterically regulating the I-domain and α7 helix through utilizing on/off switching of conformational states. I-domain is fused with EF3 and EF4 hands of calmodulin, which then regulates binding affinity to ICAM-1 through interaction with α7 helix, when the EF hands' natural ligand peptides are present. Mutant switches are being developed to alter EF hand binding specificity which, when bound to target ligand, will cause an increase in I-domain-ICAM-1 binding affinity in switch molecules. The results of these allosteric regulations highlight the potential of chimeric protein switches for design of environmentally responsive targeting agents and suggest that, through directed evolution, regulated binding to a range of novel targets could be achieved for therapeutic intervention.

Introduction

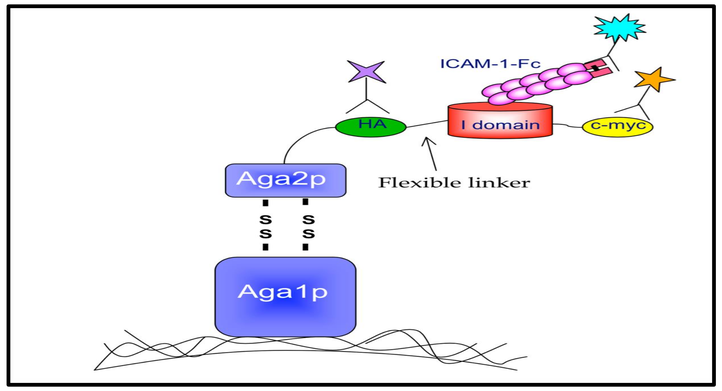
- I-domain of integrin LFA-1 mediates leukocyte adhesion to the endothelium by interaction with ICAM-1, upregulated at sites of inflammation.
- "Bell rope" model for the conformation change within I domain suggests that force applied to the α7 helix of I domain is transduced to causes structure rearrangement leading to activation.
- Understanding conformational regulation of I-domain in switch design, will further the creation of efficient environmentally responsive targeting agents. Also, giving us further insight into possible regulated binding to a range of new novel targets.



The main purpose of this research:

1. Explore the interaction of the α7 helix with the rest of the I domain to determine the mechanism of affinity regulation.
2. Develop a new chimeric I domain protein with improved affinity regulation through use of EF hand mutants bound to new novel target ligands.
3. Investigate the design of generic molecules with designable environmental triggers through directed evolution.

Main Method Used



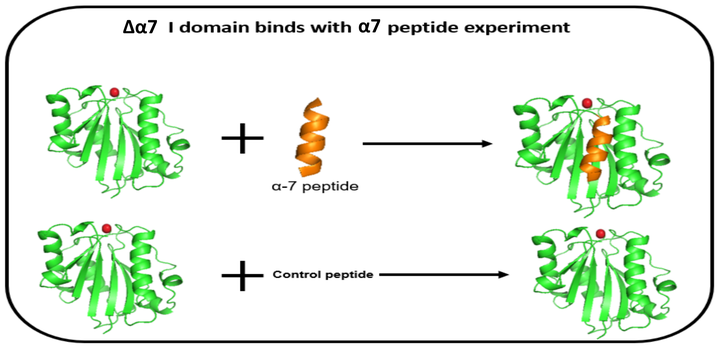
Boder, E. T.; Wittrup, K. D., Yeast surface display for directed evolution of protein expression, affinity, and stability. *Meth Enzymol* **2000,** 328, 430-44.

Yeast surface display was utilized to detect presence of expressed I-domain and binding of ICAM-1 using fluorescent tags.

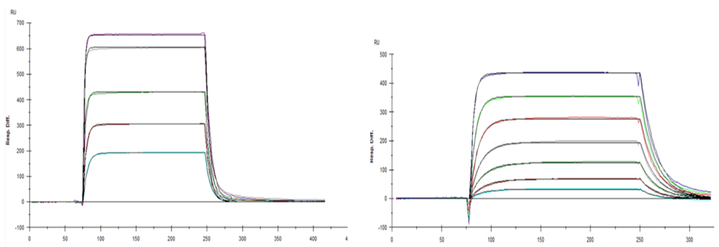
α7 helix-deleted I-domain

Design two experiments to explore the I domain affinity alteration:

1. Design an I-domain without α7 helix (Δα7 I domain)
2. Design an experiment to make Δα7 I domain bind with different peptides.



Soluble I-domain Dynamic Data



MBP-ΔA7 I domain MBP-LO I domain

I domain	k_{on} rate $M^{-1}S^{-1}x10^{-3}$	k_{off} S^{-1}	K_D uM
High affinity*	115±7	0.014±0.001	0.15±0.016
MBP-high affinity	24.8	0.063	2.58
MBP- Δ A7	148.8	0.148	1.04

* Moonsoo Jin, Gang Song, Christopher Carman, et al. Directed evolution to probe protein allostery and integrin I domains of 200,000-fold higher affinity. PNAS 2006,103,5758-5763

The Surface Plasmon resonance (SPR,Biacore 3000) data shows that soluble Δα7 I domain has similar affinity to an I-domain mutant locked into the high affinity conformation by an engineered disulfide bond.

Switch I-domain surface-displayed

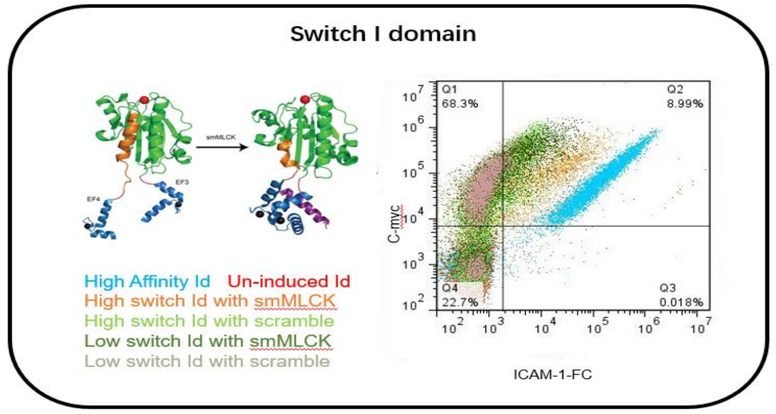
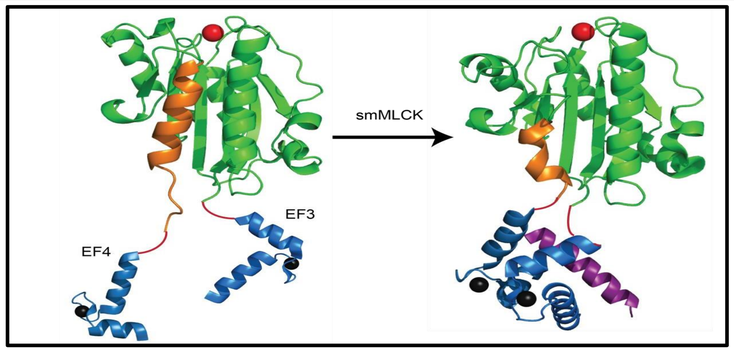


Image shows the conformational regulation of the chimeric protein switch when the EF hands are bound to their natural ligand, smMLCK. Yeast surface display data showing the detection of I-domain and EF hands of chimeric protein switches binding to target ligands.

Regulated Conformational States



When the EF hands are bound to their natural target ligand (smMLCK) it creates a allosterically regulated structural rearrangement throughout the chimeric protein switch.

Mutant Switch Screening

Through mutagenesis and directed evolution, we are engineering new switch mutants with mutated EF hands to alter peptide binding specificity. The purpose of which is to:

1. Engineer EF hands that bind to a new target ligand that will cause an increase in I-domain-ICAM-1 binding affinity in switch molecules.
2. Determine changes in I-domain binding affinity when EF hands are bound to new ligands.
3. Improve environmental response to new ligands in chimeric switch design.

We currently are screening engineered switch molecules with mutated EF hands for the purpose of increasing I-domain binding affinity through altered disruptions of the α7 helix. This also demonstrates the possibility of, through directed evolution, *regulated binding to a range of new novel targets.*

Conclusions

- Disruption of the α7 helix region of I-domain induces the high affinity conformation.
- A chimeric protein incorporating the EF hand domains from calmodulin disrupts the α7 helix in response to EF hand-binding peptide ligands.
- Alteration of peptide binding specificity data in EF hand mutants shows an increase in binding affinity in I-domain when bound to new peptides.

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