Detecting Ligand-Induced Flexibility Changes in Allosteric Proteins

Maria I. Zavodszky

Dept. of Biochemistry & Molecular Biology, Michigan State University, East Lansing, MI 48824 zavodszk@msu.edu

http://www.bmb.msu.edu/faculty/zavodszky.htm

Introduction

The functions of proteins are often modulated by binding of ligands other than their substrates. This is an important mechanism for regulating biochemical pathways. The most intriguing are the cases when the effector ligand binds to a site distant from the active site in the case of allosteric proteins. Although the observed effect is often a different conformation of the effector-bound state, the underlying cause of the altered conformation can be a change in the flexibility, leading to a change in the dynamics of the system.

Methods

The graph theoretical algorithm **FIRST**[1] was used to identify rigid and flexible regions before and after the ligand binds. The program analyzes the covalent and non-covalent bond (hydrogen bond and hydrophobic tether) network of the protein structure to identify flexible and rigid regions using constraint counting.

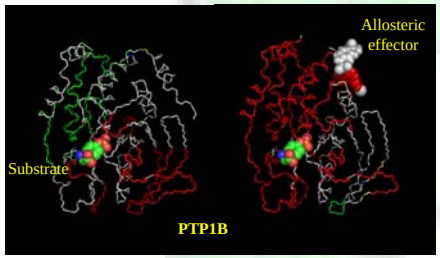
Buried waters were predicted with **PRO_ACT**[2].
Polar hydrogens were added to the protein and the water molecules using **WhatIf**[3].
Ligands were protonated using **InsightII** (Accelrys Inc. San Diego, CA).

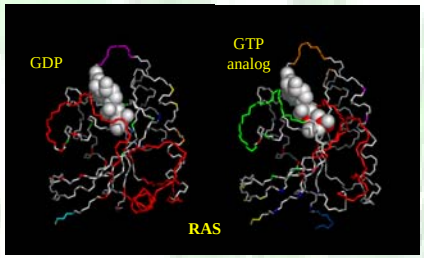
Allosteric protein pairs	Without effector	With effector
Protein tyrosine phospahatse 1b (PTP1B)	1sug	1t49
Ras G-protein	4q21	6q21

Results

Flexibility of regions around active site changed due to allosteric effector binding to a remote site. Some regions become more rigid, while others become more flexible. Backbone flexibility changes detected were relatively small and concentrated in a few regions that include the allosteric and the active site. Side-chain flexibility changes were larger in amplitude but also more diffuse.

The allosteric site and the active site were found to be coupled by flexible regions. Binding of the allosteric effector changed the size of these flexible regions (increased in the case of PTP1B and decreased in the case of RAS.

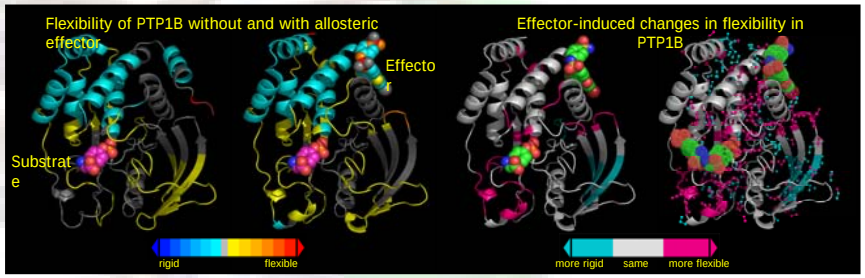




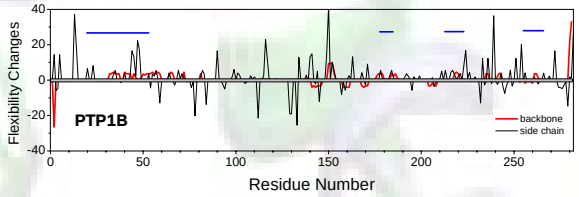
Motions are coupled within independently flexible regions identified by FIRST, each represented by a different color. For both protein pairs, the first structure is without and the second with the allosteric effector.

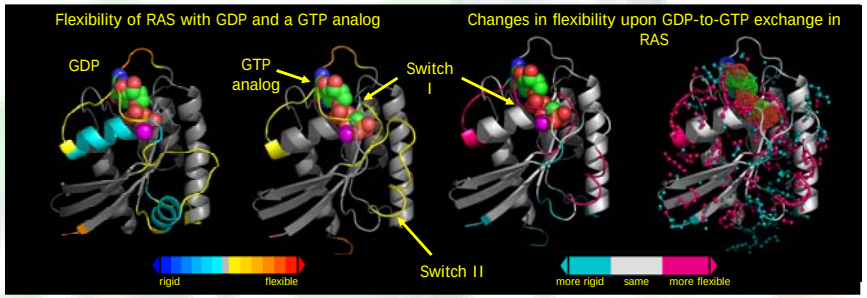
Conclusions

The flexibility changes induced by regulator binding dissipate throughout the structure, although backbone changes tend to be somewhat focused on the vicinity of the active site or interaction site. Changes in the flexibility and the size of the flexible region coupling the two sites will most likely result in an altered dynamics of the active site.

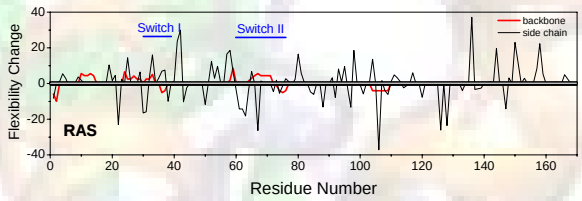


The effector inhibits PTP1B activity. Loops around the active site (blue lines below) become more flexible upon allosteric effector binding.





RAS becomes active upon exchanging GDP for GTP. Switch I and II become more flexible.



1. Jacobs DJ, Radar AJ, Kuhn LA, Thorpe MF, Proteins 44: 150, 2001
2. Williams MA, Goodfellow JM, Thornton JM., Protein Sci. 3:1224-35, 1994
3. Vriend G, J. Mol. Graph. 8:52-56, 1990

Figures created with **PyMOL** (DeLano Scientific LLC).