Comparative Binding Energy (COMBINE) Analysis of Barnase-Barstar interfacial Mutants

Running title: COMBINE analysis of barnase-barstar interfacial mutants

Abstract

Introduction

Method

Preparation of mutant complexes

The crystallographic structure of the psedo-wild type barnase-barstar complex [1] (PDB code 1b27) was used as the template to prepare most mutant complexes. The complex formed by chain A and chain D, including 213 surrounding bound water molecules, were exacted from 1b27 and Chain A was used for modeling barnase and Chain D was used for modeling barstar. Ala40 and Ala82 in barstar were mutated to cystines to restore the wild type barstar and the N-terminal methionine residue in barstar , which is far from the interface, was deleted for ease of cross-referencing. Finally, the template contains 110 barnase residues, 89 barstar residues and 213 bound water molecules.

The program WHATIF was used to add polar hydrogens to barnase, barstar and the bound water molecules in the template. His102 in barnase was protonated on Hε and His17 in barstar was protonated on Hδ. The non-polar hydrogens were added by the tLEap module of AMBER7.0. Using this modeled wild type complex structure as the template, mutations were basically done by choosing proper side chain rotamers from the InsightII rotamer library while the backbones and other residues remained (see details from Sanja).

In addition, the three mutant complexes [1] which crystallographic structures are available in PDB bank were used as templates for adding additional interfacial water molecules in the cavities created by mutations and modifying some mutants. They are K27A/barnase-D35A/barstar with the entry code of 1b2u, H102A/barnase-Y29F/barstar with the entry code of 1b3s, and K27A/barnase-T42A/barstar with the entry code of 1b2s, respectively. Comparison of interfacial water molecules in these mutants and the wild type complex indicated the following additional water molecules: WAT294 in 1b2u should be added for mutation K27A/barnase, WAT293 and WAT279 in 1b3s for mutation H102A/barnase, WAT312 and WAT319 in 1b2u for mutation D35A/barsar, and WAT298 and WAT291 in 1b3s for mutation Y29F/barstar.

All mutant complexes were prepared according to the above strategy with two exceptions: mutants with Y29F/barstar and E73W/barstar. The crystallographic structure of mutant H102A/barnase-Y29F/barstar (PDB code 1b3s) shows significant backone movement and side chain conformational change on barstar residues 28 – 30, which could not be modeled by rotamer selection. Therefore, the barstar residues 28 – 30 in complexes with mutationY29F were modeled by using 1b3s as a reference structure. For complexes with mutation E73W/barstar, the best rotamer still had serious steric clash with the neighboring residues and energy minimization by DISCOVER in InsightII was carried out on this residues (see details from Sanja). The final conformation required the removal of three water molecules WAT244, WAT302 and WAT207.

There are totally 65 mutant complexes modeled and studied in this work. The thermodynamics data (ΔG, ΔH, ΔS) mainly came from reference [2] and reference [3]. The mutations were made on 5 interfacial residues (K27, R59, E73, R83, R87, and H102) in barnase and 9 interfacial residues (Y29, D35, W38, D39, T42, W44, E76, E80) in barstar. (thermodynaics data were measured based on the wild type.)

Barnase: an extracellular ribonuclease of Bacillus amyloliquefaciens.

Three additional mutants[4]: H102K/barnase-Y29P/barstar, H102K/barnase-Y29W/barstar,

H102K/barnase-Y29P,Y30W/barstar, have crystal structures (not available in PDB) and dissocication constant data[5] .

Minimization

The all-atom AMBER 95 force field was used to obtain all the parameters of the proteins and water molecules. The tLEap module of AMBER7.0 was used to obtain the topology and coordinate files of each complex. Then the energy minimization of each complex was carried out using the Sander module of AMBER7.0 and consisted of three stages. In the first stage of 200 steps, the protein non-hydrogen atoms were restrained to their starting positions by a harmonic potential with a force constant of 32 kcal/(mol.Å^2) while the hydrogen atoms and the water molecules were unrestrained. In the second stage of 200 steps, the constraint was released from the side chain atoms of the proteins and remained on the backbone atoms only. In the third stage of 400 steps, no constraint was used at all. A non-bonded cutoff of 10.0 Å and a distance-dependent dielectric constant ($\varepsilon = r_{ij}$) was used throughout. In each stage, the first 100 steps were performed with the steepest descent algorithm and the rest of the steps were performed with the conjugate gradient method.

During the minimization, the backbone atoms of the proteins did not show observed movement and only water molecules, particularly the additional interfacial water molecules, and some side chain atoms show significant movements.

Energy decomposition by Anal

After energy minimization, the Anal module of AMBER7.0 (bug fixed) was used to calculate the Coulombic and the Lennard-Jones interaction energies between each protein residue in the complex. And a separate code (Sanja 's work) was written to extract the energy terms between each barnase residue and each barstar residue, that generated a matrix of $110 \times 89 \times 2$.

Discussion

1. Nature paper: not considered barnase.
2. Nature paper: not considered conformational changes.
3. Nature paper: the role of electr is on kinetics or thermodynamics.
4. Honig paper [6]: what indeed decides the extremely high binding constant of bn-bs.

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