Protein-protein Docking Studies of an Alpha-Lytic Protease with 2 Inhibitors using ZDOCK/RDOCK

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Abstract:

We report here the results of protein-protein docking studies of the alpha-Lytic proteinase with two proteinaceous inhibitors: turkey ovomucoid third domain (OMTKY3) and Eglin C, using ZDOCK and RDOCK. The algorithm combines the initial-stage rigid-body docking algorithm of ZDOCK based on the Fourier correlation technique with the refinement/reranking procedure of RDOCK energy-based scoring function. The results for both docking studies clearly demonstrate that the RDOCK empirical free energy scoring function of the CHARMm electrostatic interaction energy plus the Atomic Contact Energy (ACE) desolvation energy can successfully discriminate the expected enzyme-inhibitor binding mode with P1 Leu as the leading anchor residue. Furthermore, our docking study predictions provide a quantitative energetic measure that supports the experimentally determined much stronger binding strengths of Eglin c versus OMTKY3.

Introduction

Protein-protein interactions play a major role in many essential cellular processes, e.g. signal transduction, cell regulation, and immune response. To understand the molecular mechanism of biochemical processes at the atomic level, a detailed structural model of the interacting complex is required. In recent years the worldwide initiative of structural genomics projects have carried out large-scale protein structure determination in high throughput fashion, but the focus is mainly on the individual proteins. The computational algorithms capable of reliably and efficiently predicting 3D structure models of protein assemblies from the independent structures of the interacting protein partners would be invaluable tools that could complement the experimental efforts in the fields of Crystallography, NMR, and Cryo-Electron Microscopy.

In the last decade, significant advances have been made in developing **protein docking** methodologies, however, due to the complexity of the problem, it continues to be an active research area [1]. In general, adequate treatment of backbone flexibility and accurate scoring functions still prove to be challenging. In this study, we use the algorithms ZDOCK and RDOCK to validate experimental data. ZDOCK [2,3] is a fast, rigid-body, initial stage protein-protein docking algorithm that applies a pair-wise shape complementarity method that takes advantage of Fast Fourier transformation. RDOCK [4,5] is used for further refinement of complexes generated by ZDOCK via CHARMm.

Proteolytic enzymes and their many natural inhibitors are among the most studied protein complexes, different families of inhibitors and different modes of inhibition have been identified [6]. For serine proteinases, the standard mechanism, canonical inhibitors [7] have become the most thoroughly characterized systems, as a result of three decades of meticulous research on these inhibitors and their mutants in the laboratory of the late Michael Laskowski, Jr. [8,9]. The standard mechanism inhibitors are usually small, stable proteins which interact with the proteinase in a substrate-like manner. The P1 residue of the inhibitor is part of a largely exposed loop that docks into the concave proteinase catalytic site. It is interesting that in all the families of canonical inhibitors, these loop residues all have similar backbone conformation regardless of different amino acid types.

OMTKY3 is an avian egg-white protein from the third domain of Turkey ovomucoid. **Eglin C** is from the medicinal leech, it's similar in size and shape to OMTKY3, but with a different tertiary structure. While OMTKY3 has three disulfide bonds, Eglin C has no disulfide bonds, and presumably the presence of hydrogen bond interactions between two arginine residue (Arg 51, Arg53) side-chains and residues on either side of the P1 residue (Leu45) may have contributed to its thermostability.

Alpha-Lytic proteinase (ALP) is a serine proteinase from the soil bacteria Lysobacter enzymogenes. It belongs to an interesting class of proteins that were synthesized in the cell with a covalently attached pro region which is necessary for their proper folding. In the SCOP classification it belongs to the Superfamily of Trypsin-like proteinases.

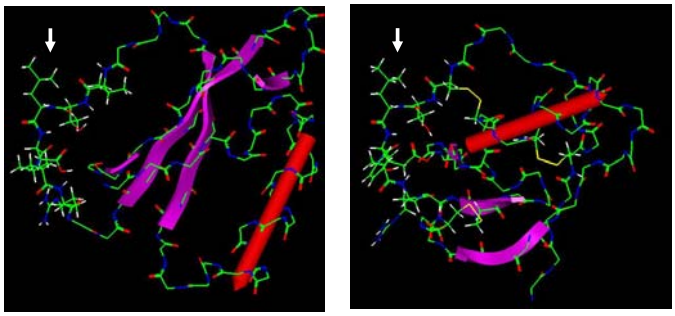

Figure 1 Eglin C (left) and OMTKY3 (right). Shown with the exposed loop segments in the same orientation. The white arrow points to the P1 Leu residue.

Methodology*

We have performed protein-protein docking of ALP with two natural inhibitors, Eglin C and OMTKY3, using the ZDOCK & RDOCK (Accelrys Software). In both docking studies, the structure of the ALP used is taken from PDB 1GBK, in which the structure was solved in a complex with a small peptide boronic acid inhibitor. The structure for Eglin C is taken from 1ACB, and the structure for OMTKY3 is from 1CHO, both were complexed with alpha-chymotrypsin. **Figure 1** display the two inhibitors side by side with the exposed loop segments in the same orientation, the P1 residue is Leucine in both molecules.

ZDOCK for protein-protein docking included using a filter feature to specify the residues involved in the binding interface since we expect the catalytic site of the proteinase and the exposed loop region of the inhibitor to be involved. In the ALP-Eglin C docking, the catalytic histidine (His57) of ALP and the P1 residue (Leu45) are specified for filtering. In the ALP-OMTKY3 docking, the same catalytic histidine and the P1 residue (Leu18) are specified for filtering. **RDOCK** refinement was performed on the top 50 poses of the filtered ZDOCK output

Results and Discussion

Figure 2 and 3 illustrate the predicted proteinase-inhibitor complex conformations. For both studies the top ranking poses form clusters that reveal the correct binding mode. Both inhibitors are wedge-shaped, with the exposed loop region as the front, narrow edge of the wedge. Among the docked poses in the cluster, the loop residues on both side of the P1 residue tend to exhibit much smaller backbone RMSD compared with the residues on the wider part of the molecule. Protein side-chain movements among the binding interface residues are observed after the RDOCK refinement, while the backbone conformation remains largely unchanged. Several intermolecular hydrogen bonds consistently form between the exposed loop and the active site region of the proteinase in both complexes, forming a short segment of antiparallel beta-sheet.

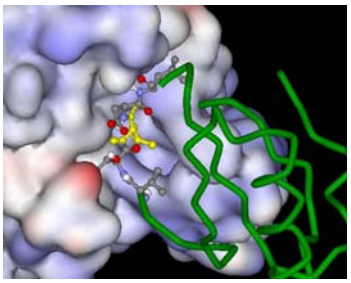

Figure 2. Top rank ALP-Eglin C complex, ZDOCK pose #43. ALP is rendered as a solvent accessible surface, colored with electrostatic potential. Eglin C loop residues Val43, Thr44, Leu45 (yellow), Asp46, and Leu47 are in ball-and-stick and the rest of Eglin C rendered as tube.

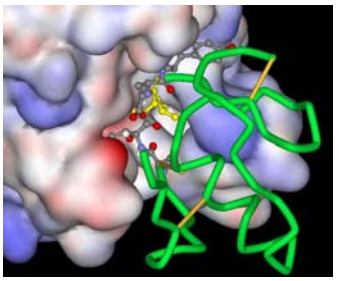

Figure 3. Top rank ALP-OMTKY complex, ZDOCK pose #5. ALP is rendered as a solvent accessible surface. OMTKY3 loop residues Cys16, Thr17, Leu18 (yellow), Glu19, and Tyr20 are in ball-and-stick and the rest of OMTKY3 rendered as tube.

Remarkably, for the ALP-Eglin C complex, the first 25 ranked poses out of the 50 RDOCK predicted poses are clustered around the common binding mode. For the Alp-OMTKY3 complex, such clustering are only seen with the first 8 ranked poses out of the 50 RDOCK predicted poses. This finding for *a*-lytic protease complexes is consistent with computational free energy calculations conducted for *a*-chymotrypsin-inhibitor complexes, where it was found that desolvation interactions were the major contributors to the overall stability of the complexes.

Conclusions

The results for both docking studies clearly demonstrate that the RDOCK scoring function can successfully discriminate the expected enzyme-inhibitor binding mode with P1 Leu as the leading anchor residue in both systems. This correct binding mode is identified as the # 1 predicted pose in both systems. Moreover, for the ALP-Eglin C complex, the top-ranking poses form a very large size cluster all with the correct binding mode, and all with very favorable ACE and electrostatic energy scores. The docking study predictions provide a quantitative energetic measure that supports the experimentally determined much stronger binding strengths of Eglin C versus OMTKY3 for alpha-Lytic proteinase.

References

Publication of results: Qasim et al., "Despite having a common P1 Leu, eglin C inhibits alpha-lytic proteinase a million-fold more strongly than does turkey ovomucoid third domain." Biochemistry. 2006 Sep 26;45(38):11342-8. *** see for detailed methodology**

ZDOCK/RDOCK products from Accelrys (www.accelrys.com)

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