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Research Topic

The identification of binding specificity of dual specificity phosphatases (DSP) via docking experiments on the Grid.

Abstract

The purpose of this experiment is to identify the binding specificity of dual specificity phosphates family members via docking experiments on the Grid. The dual specificity phosphatases (DSP) is a class of enzyme that plays a significant role in the regulation of cell growth . The slingshot (SSH2) protein is a member of the dual specificity phosphatases that is responsible for the regulation of cofilin activity, which plays a critical role in the development of Alzheimer's disease and cancer. Even though a list of high potential inhibitors for SSH2 proteins has been identified in previous experiments, the binding specificity of these potential inhibitors have not been entirely defined. It is of great interest to identify the binding specificity of these potential inhibitors because it gives insights into the molecular interactions of chemicals and the SSH2. In this experiment, the binding specificity DSP family member PRL-3 and MKP-6 was screened against ligands from ZINC database using the molecular docking software DOCK 6. Top binding ligands for both PRL-3 and MKP-6 were identified and ranking results were compared with SSH-2. According to consensus ranking, top 3 highest binding compounds for PRL-3 were as follows: ZINC06662815, ZINC6645916, and ZINC66431804. Top 3 highest ranking compounds for MKP-6 were ZINC00310164, ZINC00074595 and ZINC02384698 according to energy score alone.

Introduction

The dual specificity phosphatases (DSP) belong to the Protein Tyrosine Phosphatases (PTP) super family. The main function of DSP members is the selective dephosphorylation of phospho-threomine/serine and phospho-tyrosine residues. Some DSP proteins are also classified as mitogen-activated protein phosphatases (MKP) because they are responsible for the dephosphorylation of threonine and tyrosine residue in MAP kinase. This dephosphorylation of either threonine or tyrosine residue leads to the complete inactivation of MAP kinase which affects the signal transduction pathway. The DSPs that are responsible for MAP inactivation display high substrate specificity towards MAP kinases due to the tight binding in its N-terminal regulatory domain.

Another important member of the DSP family is the slingshot-2 (SSH2) protein which plays a critical role in the regulation of cofilin activity. Because cofilin is responsible for the promotion of actin assembly during cell proliferation, the inactivation of cofilin by SSH-2 promotes actin disassembly which is directly related to the development of Alzheimer's disease and cancer. Therefore, the development of SSH-2 inhibitor may be used for clinical and pharmacological treatments of these diseases. Finding the specificity of these inhibitors on other DSPs can give important insight into the interactions of chemicals with SSH-2 active site which can be useful in predicting possible side effects of these inhibitors.

The major function of DSPs is defined by their ability to dephosphorylate particular substrates. According to the model catalysis in Protein Tyrosine Phosphatases (PTP), cystine acts as nucleophile in the active site which leads to the formation of thiophosphate intermediate. Aspartic acid then donate proton to the leaving group and, as water attacks the phospho-enzyme intermediate, enzyme is regenerated. This mechanism is responsible for the dephosphorylation of threonine and tyrosine. However, because the DSPs' active sites are shallower than other PTPs, the DSPs are expected to

display less stringent phospho-amino acid specificity.

The gene expression of DSP is activated by growth factors as well as cellular stresses. As these enzymes are expressed predominantly in tissues such as brain, heart, skeletal muscle and kidney, the discovery of DSP inhibitors can be used to treat diseases in these areas. Specifically, since SSH2 is known to be involved in regulating the migratory potential of cancer, the discovery of SSH2 specific inhibitor can be used as possible treatment for brain cancer.

Methods

The binding affinity of potential SSH-2 inhibitors was determined through virtual screening experiments using DOCK 6 software on the grid. Top 1% of the ZINC database was screened against receptor molecules in Grid Based Energy Screening, a fast screening method that took into account Van der Waals and electrostatic forces to generate an energy score. ZINC database ligands, input files and resources were sent as input into the master cluster. Input files and requests to launch DOCK MPI were delivered from the master cluster to remote clusters through Dock Services and opal-op toolkits. Input files were then sent to scheduler on each remote cluster to launch DOCK MPI and generate screening results. Upon completion all slices, screening results from each remote clusters were retrieved back to the master cluster to generate Energy Score ranking. All of the top 1% of ligands from ZINC database were then re-screened using AMBER scoring method, a more precise method that took into account induced-fit and molecular dynamic force field. AMBER preparation was done before AMBER screening to generate all AMBER compatible input files. Because AMBER preparation step was not done in parallel, AMBER screening method was relatively time-consuming. AMBER screening was done using a more precise method in which single rigid portion of the molecule was oriented into the binding site and the rest of the molecules were then added to create flexible docking. Because AMBER took into account solvation energies, molecular dynamic force field and induce fit, more precise screening results could be generated. Upon completion of AMBER screening, results from remote

clusters were retrieved back into the master cluster to generate AMBER score ranking. Consensus ranking was calculated by taking the sum of Energy based ranking and AMBER ranking. Clusters used in this experiment included Tea, Cafe, Rocks-200, Rocks-153, Sakura, Hallasan KISTI, Pragma lzu and Ocikbpra. Average time used per molecules was 6 days for Grid-based Energy Screening and 2 weeks for AMBER screening.

Results

Top 10 most specific ligands for PRL-3 are listed in Table 1. The highest ranked compound ZINC06662815 was originally ranked number 7 on the consensus ranked list but the top 6 molecules did not receive an AMBER score. These top 6 molecules were removed from following list due to the possibility that lack of AMBER result would skew the calculation for consensus ranking and give inaccurate results.

Table 1. Consensus ranked results for PRL-3

Ra	ZINC ID	Energy Rank	
1	06662815	146	192
2	06645916	346	3
3	06431804	403	35
4	05093617	453	1
5	03264695	361	93
6	02649005	414	104
7	03264693	410	167
8	02655615	332	267
9	02107940	279	435
10	02650299	502	245

According to consensus ranking, top 4 most specific ligand for PRL-3 were ZINC06662815, ZINC6645916 and ZINC06431804. However, ZINC06431804 received an AMBER score of –158571642880.0, which was 10 orders of magnitudes lower than the scores of other ligands.

Visualization using Chimera showed multiple ligand-receptor interactions for ZINC 06431804, making the abnormally low AMBER score unreliable. The 4th highest ranking ligand ZINC05093617 showed similar problem as ZINC06431804, similarly making its consensus ranking invalid.

PRL-3 binding specificities for high potential SSH-2 inhibitors are listed in table 2.

Table 2 Comparing consensus results from SSH-2 and PRL-3

ZINC ID	SSH-2	PRL-3	Difference (PRL-3 - SSH-2)
05260817	1	NA	NA
03869281	2	139	137
04543673	3	7275	7272
02384698	4	2115	2111
03869935	5	2044	2039
04521532	6	247	241
04543675	7	1851	1844
02522549	8	378	370
04652509	9	6609	6600
02637978	10	6275	6265

By comparing the consensus results for SSH-2 and PRL-3, binding specificity for potential SSH-2 inhibitors can be predicted. As seen from the results above, ZINC04543673 showed the greatest differences between PRL-3 and SSH-2, which reveals greatest potential as SSH-2 inhibitor. This compound, however, only showed a difference of 852 in consensus results between SSH-2 and VH1, which creates the possible problem that even though it binds to SSH-2 with much higher affinity than

PRL-3, it could not be used as useful SSH-2 specific inhibitor because it binds to VH1 (and possibly some other DSP members) with similarly high affinity. ZINC02637978, on the other hand, shows the second highest difference between PRL-3 and SSH-2 while also showing a high difference of 17268 between VH1 and SSH-2. This difference makes ZINC02637978 a more hopeful SSH-2 specific inhibitor than ZINC04543673 because it binds to both VH1 and PRL3 with equally low affinity.