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Identification of inhibitors against the potential ligandable sites in the active cholera toxin

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ABSTRACT

The active cholera toxin responsible for the massive loss of water and ions in cholera patients via its ADP ribosylation activity is a heterodimer of the A1 subunit of the bacterial holotoxin and the human cytosolic ARF6 (ADP Ribosylation Factor 6). The active toxin is a potential target for the design of inhibitors against cholera. In this study we identified the potential ligandable sites of the active cholera toxin which can serve as binding sites for drug-like molecules. By employing an energy-based approach to identify ligand binding sites, and comparison with the results of computational solvent mapping, we identified two potential ligandable sites in the active toxin which can be targeted during structure-based drug design against cholera. Based on the probe affinities of the identified ligandable regions, docking-based virtual screening was employed to identify probable inhibitors against these sites. Several indole-based alkaloids and phosphates showed strong interactions to the important residues of the ligandable region at the A1 active site. On the other hand, 26 top scoring hits were identified against the ligandable region at the A1 ARF6 interface which showed strong hydrogen bonding interactions, including guanidines, phosphates, Leucopterin and Aristolochic acid VIa. This study has important implications in the application of hybrid structure-based and ligand-based methods against the identified ligandable sites using the identified inhibitors as reference ligands, for drug design against the active cholera toxin.

1. Introduction

The functionally active cholera toxin is a heterodimer of the A1 subunit from the *Vibrio cholerae* holotoxin and the ADP Ribosylation Factor 6 (ARF6) from the human host cytoplasm (Kaper et al., 1995; Chaudhuri and Chatterjee, 2009; Galloway and Van Heyningen, 1987; Jobling and Holmes, 2000; Lee et al., 1991), which is responsible for the ADP-ribosylation that causes the immense loss of water and electrolytes from the host cells. Structural and experimental studies on the active cholera toxin provide clear insight on the mechanism of A1 activation following ARF6 binding, along with the substrate interactions at the active site (O'Neal et al., 2005a). Experimental studies show that the binding of ARF6 to A1 is necessary for activation of the latter by exposure of the active site residues following a conformational change in its active site loop (O'Neal et al., 2005a). The ADP-ribosyltransferase activity of the activated A1 triggers a downstream cascade leading to cAMP build-up, and a subsequent loss of water and ions from the host cell.

Although proteins often contain numerous pockets, the presence of a pocket does not necessarily imply that it is druggable. The "druggability" of a protein pocket refers to its ability to bind to small drug-like molecules which modulates the disease-causing function of the protein (Edfeldt et al., 2011; Hopkins and Groom, 2002). Over the recent past, the "druggability" of a target has been used simply to refer to the ability of a protein to interact with small molecules with high binding affinity (Egner and Hillig, 2008). "Druggability" in such cases becomes a misnomer, and calls for the use of a more suitable nomenclature – "ligandability", as suggested by many authors (Edfeldt et al., 2011). The ligandability of a protein can be predicted by various methods, such as energy-based methods based on the energy of interaction between a target and various chemical probes (Henrich et al., 2010; Laurie and Jackson, 2005; Wade and Goodford, 1993). Computational solvent mapping, often used to predict "druggable" sites in proteins, is based on identifying "consensus" residues that bind to organic solvent probes (Henrich et al., 2010; Landon et al., 2007; Dennis et al., 2002). Here, "druggable" sites refer to ligandable sites which have a potential to bind to molecules having drug-like properties. The "druggability" of a consensus site depends

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Table 1
Number of drug-like compounds retrieved from PubChem, based on probe affinity of ligandable sites 1 and 2 (LBS1 and LBS2 at the A1–ARF6 PPI and the A1 active site, respectively).

Probe	Probe affinity for ligandable site	Chemical nature of compounds selected, based on probe affinity	Number of screened drug-like compounds selected on the basis of chemical nature of probe
CH4	LBS1, LBS2	Alkanes	17
CL	LBS1	Chlorides	524
O	LBS1	Carbonyls	227
OP	LBS1, LBS2	Phosphates	1817
S	LBS1	Sulphur containing compounds	378
SI	LBS1, LBS2	Silicon containing compounds	44
NE	LBS1, LBS2	Guanidines	699
N	LBS1, LBS2	Alkaloids (representative compounds)	111
		Nitrogen containing compounds	1105
Total number of compounds screened against LBS1			4291
Total number of compounds screened against LBS2			3792

on the population of the solvent cluster it binds. A consensus site is considered to be "druggable", when it binds to a high population cluster, and is also simultaneously associated with neighbouring clusters of low population (Hall et al., 2012). Sites in proteins that satisfy these criteria have been found to bind drug-like ligands (Hall et al., 2012).

While there have been numerous efforts to target toxin entry by preventing binding of the B subunits to the host cell as a means of treating cholera (Branson and Turnbull, 2013), the druggable potential of the active toxin has been very little explored. While many authors suggest the inhibition of the A1 active site as an effective means of cholera treatment (Chaudhuri and Chatterjee, 2009; Fan et al., 2004) thus making it a possible drug target, the ligandability or chemical affinity of the active site or other target sites in the active toxin complex have been overlooked. Therefore, in this study, we have proceeded to identify the potential ligandable sites in the active toxin, and explored the affinity of these sites for different chemical probes, for ligand-based and structure-based design of inhibitors against cholera. The other neglected drug target in the heterodimeric toxin could be the PPI (protein–protein interface) of A1 and ARF6. The fact that the inhibition of many PPIs have proved effective in disease treatment (Wells and McClendon, 2007; Jubb et al., 2012; Cochran, 2000), places emphasis on exploring the ligandability of the toxin PPI.

In this study, we used an energy-based approach for calculation of ligand binding sites, based on the affinities of the target for different chemical probes (Ghersi and Sanchez, 2009). Computational solvent mapping with organic solvents was employed to identify probable "druggable" regions in the toxin. By correlating the predicted ligand binding sites with the "druggable" sites identified by computational solvent mapping, we could identify two potential ligandable sites in the toxin, which can probably bind drug-like molecules with high affinity. The ligandable sites that were identified had importance in terms of toxin function, as later discussed. Based on their binding affinity for the different probes, we performed an *in silico* docking based screening of drug-like compounds against the identified ligandable sites. The results indicate that indole-based alkaloids and phosphates could act as inhibitors of the A1 active site. 26 compounds were identified against the toxin PPI, which exhibited strong hydrogen bonding interactions to key residues involved in the A1–ARF6 interaction. These compounds can be treated as reference ligands for ligand-based virtual screening of inhibitors against the cholera toxin.

2. Materials and methods

2.1. Homology modelling and model validation

The sequences of the cholera toxin A1 subunit and the human ARF6 (UniProt entries P01555 and P62330, respectively) were retrieved (The UniProt Consortium, 2013). A comparative model of the A1–ARF6 (Mg-GTP bound ARF6) heterodimer was constructed using the partial structure of the active toxin heterodimeric complex as the template (PDB ID: 2A5D) (O'Neal et al., 2005b; Berman et al., 2000, 2003), using Modeller 9.10 (Šali and Blundell, 1993). Residues 1–11 of the human ARF6 and 188–194 of the A1 were subjected to loop refinement.

Model validation was performed to check structural and functional conformity. Structure validation was done by analysing the Ramachandran plot (Chen et al., 2009), while stereochemical and bonding parameters were used to identify geometrical distortions (Arnold et al., 2006; Laskowski et al., 2005a). Functional validation was performed to confirm the enzymatic and catalytic potentials of the active cholera toxin (Laskowski et al., 2005b).

2.2. Prediction of toxin pockets and ligand binding sites

A probe radius of 1.4 Å was used for prediction of protein pockets (Dundas et al., 2006). The ligand binding sites were predicted based on the energy of interaction between the target and 18 probes (Ghersi and Sanchez, 2009). The molecular interaction fields were calculated using EasyMIFs. The binding sites were predicted by the agglomerative hierarchical clustering algorithm of SiteHound, using average linkage. For each probe, the first four predicted binding sites were studied. The residues which showed binding to a large number of probes (9 or above) were identified as ligand binding sites. The 18 probes used in the study for predicting ligand binding sites were: aliphatic probes (CH1, CH2, CH3), methane (CH4), methyl carbon (CMET), aromatic carbon (CR1), sulphur (S), chlorine (CL), oxygen (OMET), silicon (SI), phosphate oxygen (OP), carbonyl (O), carboxyl (OM), hydroxyl (OA), water (OW), peptide nitrogen (N), arginine nitrogen (NE), aromatic nitrogen (NR) (Ghersi and Sanchez, 2009).

2.3. Computational solvent mapping of organic solvents

Computational solvent mapping was performed using 16 organic solvents (acetaldehyde, acetamide, acetone, acetonitrile, benzaldehyde, benzene, cyclohexane, dimethyl ether, ethane, ethanol, isobutanol, isopropanol, methylamide, *N*,*N*-dimethylformamide, phenol and urea) to the toxin heterodimer (Ngan and Bohnuud et al., 2012). The consensus binding sites were studied with PyMol.[1] For the site to be ligandable with drug-like molecules, we considered a criteria where the consensus site had to bind to a cluster of over 10 probes clustered within a radius of 4 Å, and had to

[1] The PyMOL Molecular Graphics System, Version 1.6.0.0, Schrödinger, LLC.