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Research article

Molecular dynamics and high throughput binding free energy calculation of anti-actin anticancer drugs—New insights for better design

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ABSTRACT

Actin cytoskeleton plays an important role in cancerous cell progression. Till date many anticancer toxins are discovered that binds to different sites of actin. Mechanism of action of these toxins varies with respect to the site where they bind to actin. Latrunculin A (LAT) binds closely to nucleotide binding site and Reidispongiolide binds to the barbed end of actin. LAT is reported to reduce the displacement of domain 2 with respect to domain 1 and allosterically modulate nucleotide exchange. On the other hand Reidispongiolide binds with the higher affinity to actin and competes with the DNaseI binding loop once the inter-monomer interaction has been formed. Evolving better actin binders being the aim of this study we conducted a comparative molecular dynamics of these two actin-drug complexes and actin complexed with ATP alone, 50 ns each. High throughput binding free energy calculations in conjugation with the high-throughput MD simulations was used to predict modifications in these two renowned anti-actin anticancer drugs for better design. Per residue energy profiling that contribute to free energy of binding shows that there is an unfavourable energy at the site where Asp157 interacts with 2-thiazolidinone moiety of LAT. Similarly, unfavourable energies are reported near macrocyclic region of Reidispongiolide specifically near carbons 7, 11 & 25 and tail region carbons 27 & 30. These predicted sites can be used for modifications and few of these are discussed in this work based on the interactions with the binding site residues. The study reveals specific interactions that are involved in the allosteric modulation of ATP by these two compounds. Glu207 closely interacting with LAT A initiates the allosteric effect on ATP binding site specifically affecting residues Asp184, Lys215 and Lys336. RGA bound actin shows high anti-correlated motions between sub domain 3 and 4. Unlike LAT A, Reidispongiolide induces a flat structure of actin which definitely should affect actin polymerisation and lead to disassembly of actin filaments.

1. Introduction

Actin cytoskeleton plays a fundamental role in the process of cellular transformations (Janmey and Chaponnier, 1995). Cancerous cell shows change in the morphology of the cell, migration and invasion and this aggressive transformation requires the cytoskeleton machinery to alter its dynamics (Weed and Parsons, 2001; Pollard and Borisy, 2003; Button et al., 1995; Lambrechts et al., 2004). Actin cytoskeleton plays an important role in cell angiogenesis and cancerous cell migration. Especially, dynamics of polymerisation and depolymerisation of actin cytoskeleton plays an important role in this transformation (Jordan and Wilson, 1998; Wittekind and Neid, 2005; Harlozinska, 2005; Sahai., 2005). Therefore, actin targeting phytochemicals are used as anticancer agents, specifically stopping angiogenesis and cell proliferation (Hayot et al., 2006; Fenteany and Zhu, 2003; Jordan, 1998; Cherrington et al., 2000). Latrunculin A (LAT), a compound isolated from red sea sponges, binds to monomeric actin and prevents actin polymerisation thus disrupting the actin cytoskeleton (Spector et al., 1983; Coué et al., 1987; Ayscough et al., 1997; Belmont et al., 1999; Safer et al., 1997). LAT also affects nucleotide exchange like

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many other proteins that bind to nucleotide binding region (Rennebaum and Caflisch, 2012). Conformational/Structural changes of actin caused by LAT are studied in detail using molecular dynamics studies (Mohammad Khanfar and Diaa Youssef, 2010), it is reported that binding of LAT hinders the conformational transition related to actin polymerization. In particular, the presence of LAT at the interface of the two major domains of monomeric actin reduces the correlated displacement of Domain 2 with respect to Domain 1, reduced intradomain correlation of Domain 2 in the presence of LAT, indicates that Domain 2 moves to a lesser extent as a rigid body relative to Domain 1 in the LAT complex (Rennebaum and Caflisch, 2012) Substitutions at C-17 hydroxyl and thiazolidinonenitrogen of LAT has proven to improve the actin-binding affinity which was earlier predicted by QSAR studies (Khanfar et al., 2010; Klenchin et al., 2003). Another set of modulators that affect actin dynamics are compounds that bind to barbed end of actin. Crystallographic studies of the compounds that bind to the barbed end provides important details of the molecular interactions that confer their high affinity for actin (Allingham et al., 2004; Klenchin et al., 2005a; Allingham et al., 2005; Hirata et al., 2006; Rizvi et al., 2006; Allingham et al., 2006). Barbed end-binding macrolides typically possess a structurally variable hydrophobic macrocycle (the "ring") and a long flexible aliphatic side chain (the "tail") with conserved stereochemistry that terminates with a highly conserved *N*-methyl-vinylformamide (MVF) moiety. Structural alignment studies have shown crucial pharmacophoric regions, specifically in the tail region to improve its binding affinity for actins. Of the many macrocyclic compounds that bind to the barbed end, special interests is of Reidispongiolide (RGA) which is related to macrocyclic lactone constituents of new Caledonian marine sponges Reidispongiacoerulea; RGA binds to actin with high affinity and exhibit potent cytotoxicity against numerous human cancer cells (Zhang et al., 1997; Guella et al., 1989; Behrmann et al., 2012). In this study we have taken two anti-actin anticancer molecules that bind to two contrasting sites in actin. LAT that binds near nucleotide binding site and RGA that binds to barbed end of actin were simulated using molecular dynamics to estimate interaction free energies. Per residues' contribution to the binding energy was used to predict modification sites in these molecules that can be used for making better actin binders. Extensions to these studies post different simulations studies were conducted to point out residue level information on the allosteric modulation on ATP hydrolysis.

2. Materials and methods

2.1. Complexes used for simulation

The coordinates of the LAT, RGA bound to actin were downloaded from PDB database, 1ESV (Morton et al., 2000) and 2ASM (Allingham et al., 2005) respectively. F-actin bound to ADP alone was obtained from Actin-tropomyosin and Myosin complex (Pfaendtner et al., 2009). The over all RMSD between the superimposed structure of the actin complexed with LAT and RGA was 0.7 Å. Since the D-loop is highly flexible its residues are not present in the PDB files this region was modelled using the PDBID: 3DAW using SWISS-MODEL as in previously published MD studies of-actin (Zheng et al., 2007; Dalhaimer et al., 2008). The actin coordinates complexed with LAT and RGA and ATP alone were simulated for 50 ns each. Post simulation studies were conducted to predict better binders, residues showing key interactions, region of drug for modification and allosteric effects. For convenience the two simulations will be referred as Actin-LAT for actin complexed with LAT, Actin-RGA for actin complexed with RGA and F-actin for actin complexed with ADP.

2.2. Molecular dynamics

Partial atomic charges for LAT, RGA and ATP were a assigned based on the AM1-BCC method (Jakalian et al., 2002; Wang et al., 2006) using the antechamber program of Amber Tools (Case et al., 2012). Amber ff12SB and GAFF force fields were used to generate the topology and coordinate files for the protein and ligand respectively. The topologies file for Gromacs were prepared using the programme Topolbuild, developed by Bruce D. Ray (personal communication). MD simulations were performed in isothermal-isobaric conditions in a periodic cubic box with an edge length of approximately 8.2 nm. The protein was placed in a cubic box containing water molecules using explicit solvent SPC/E model water molecules around protein complex and its charge was neutralized using K+ ions. Of the four models (TIP3P, TIP4P, SPC, and SPC/E), SPC/E's translational diffusion constant and the rotational correlation time are the closest to the experiment values (Wakai et al., 2014), therefore the SPC/E model was employed. Then by a 200 ps constant volume simulation with 1 fs time step, the system was heated to 300 K. A 500 ps NPT simulation with 2 fs time step equilibrated the pressure to 1 atm. All heavy atoms were position restrained with the force constant of 1000 kJ/(mol nm 2) in both the simulations. A 1 ns simulation with a time step of 2 fs caused the position restraint to be slowly removed. The Berendsen algorithm was used to regulate both temperature and pressure (Berendsen et al., 1984). MD production simulations of the whole system for 50 ns, using 0.002 ps time step was finally performed. The electrostatics were calculated using PME method with a real space cut-off of 10 Å, using order of 4 and a relative tolerance between long and short range energies of 10^{-} (Lambrechts et al., 2004). Short range interactions were evaluated using a neighbour list of 10 Å and the Lennard-Jones (LJ) interactions and the real space electrostatic interactions were truncated at 9 Å. The V-rescale thermostat was used to maintain the temperature; hydrogen bonds were constrained using LINCS algorithm. Post simulation analysis in this study was carried out using *g_dist, g_mindist, g_sgangle*and *g_rms* provided in the Gromacs package. Visualisation studies were done using VMD 1.9 and Chimera 1.7.

2.3. Twist angle, Motional correlations and Torsion angle analysis

Twist angle between domain 1 and domain 2 were calculated using the same procedure as described in previous reports (Pfaendtner et al., 2010). Each MD snapshot was superimposed onto the monomeric ADP-Actin structure (PDB ID 1J6Z) using do_multiprot (Oda and Mae'da, 2010) and DCC algorithm (Shatsky et al., 2004) was used to derive the axis of rotation to calculate twist angle. WORDOM was used to calculate the cross-correlation analysis of the atomic fluctuations from the simulations (Seeber et al., 2011). *g_sgangle* from Gromacs package was used to compute the dihedral angle distribution which was plotted to study the twist angle of different simulations.

3. Results

3.1. Free energy of binding of LAT and RGA after simulations reveals RGA as the better binder

Free energy of binding calculated for the two simulations, LAT-actin and RGA-actin shows that RGA binds to actin with a higher affinity than that of LAT. The average binding energy of RGA to actin (-204.17 ± 19.1 kJ/mol) is ~2 fold better than LAT to actin (-88.22 ± 22.3 kJ/mol). The per residue energy contribution to the binding energy of RGA is 117.64 ± 0.1454 kJ/mol, which is 1.5 times better than LAT (-79.1 ± 0.1439). It is