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Docking, molecular dynamics and QM/MM studies to delineate the mode of binding of CucurbitacinE to F-actin

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ABSTRACT

CucurbitacinE (CurE) has been known to bind covalently to F-actin and inhibit depolymerization. However, the mode of binding of CurE to F-actin and the consequent changes in the F-actin dynamics have not been studied. Through quantum mechanical/molecular mechanical (QM/MM) and density function theory (DFT) simulations after the molecular dynamics (MD) simulations of the docked complex of F-actin and CurE, a detailed transition state (TS) model for the Michael reaction is proposed. The TS model shows nucleophilic attack of the sulphur of Cys257 at the β-carbon of Michael Acceptor of CurE producing an enol intermediate that forms a covalent bond with CurE. The MD results show a clear difference between the structure of the F-actin in free form and F-actin complexed with CurE. CurE affects the conformation of the nucleotide binding pocket increasing the binding affinity between F-actin and ADP, which in turn could affect the nucleotide exchange. CurE binding also limits the correlated displacement of the relatively flexible domain 1 of F-actin causing the protein to retain a flat structure and to transform into a stable "tense" state. This structural transition could inhibit depolymerization of F-actin. In conclusion, CurE allosterically modulates ADP and stabilizes F-actin structure, thereby affecting nucleotide exchange and depolymerization of F-actin.

1. Introduction

Actin is one of the major cytoskeletal proteins required to maintain the structure and shape of the cell. Multicellular organisms contain multiple actin genes, encoding protein isoforms with different functions such as muscle contraction, cytokinesis, cell mobility and the control of cell shape and polarity [1]. Actin monomers (G-actin) can polymerize to form filamentous actin (F-actin) and F-actin undergoes depolymerization by hydrolysis of ATP [2]. This dynamic process produces actin bound to ADP-Pi and ATP in both G-actin and F-actin forms [3]. The two major types of actin that are abundantly found are, α-actin, which is associated with contractile structures, and β-actin that specifically controls cell growth and migration. Many classes of phytochemicals are known to modulate different cellular functions including cell morphogenesis, movement and division by directly interacting with actin. Latrunculin (LAT), cytochalasin, jasplakinolide and phalloidin inhibit α-actin polymerization [4,5]. Azadirachtin is reported to bind to β-actin and inhibit polymerization, in a similar mode to that of LAT binding to actin [6]. A recent study has shown that a different chemical form of Cucurbitacin, a triterpenoid, bind to actin and cause formation of actin aggregates [7]. One specific form, CurE, irreversibly binds to F-actin forming a covalent bond between its Michael Acceptor (MA) and Cys257 of the subdomain 4 of F-actin [8]. This covalent binding inhibits actin depolymerization in a manner unlike that of any other known phytochemicals (phalloidin and jasplakinolide) [5]. Since, in all available experimental structure of actin, Cys257 is found to be deeply buried, the stable binding of CurE to Cys257 seems possible only if F-actin undergoes considerable conformational change so as to accommodate the tetracyclic scaffold of CurE. Latrunculin A (LAT A) binds to actin in the cleft between subdomains 2 and 4 of actin and inhibits adenine nucleotide exchange on actin [9]. Also, LAT A binds close to the adenine nucleotide binding site and this might limit the flexibility of the cleft and trap the nucleotide. Cys257 is also close to the nucleotide binding site; therefore, the covalent binding of CurE to Cys257 could also affect the conforma-

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tion of the nucleotide binding pocket. Considering all these factors we have conducted different simulation experiments using techniques such as MD, QM/MM and DFT to predict the bond forming binding mode of CurE and its impact on F-actin structure.

2. Materials and methods

2.1. Generating initial binding modes of CurE

The 3D structure of CurE was docked against the F-actin structure (PDB ID: 2ZWH) using Autodock 4.2. A grid was set on F-actin centering on the alpha carbon of Cys257 with grid spacing dimensions of $X = 58$ Å, $Y = 54$ Å and $Z = 52$ Å and 1000 GALS runs were executed to explore all possible conformations of CurE on domain 2 of F-actin. By analyzing the distance between MA and the S atom of Cys257, certain F-actin-CurE complexes were selected for simulation studies.

2.2. MD simulation experiments

Selected F-actin-CurE complexes were simulated for 20 nano seconds (ns) each using Gromacs 4.5.4 package [13,14] with Amber ff12SB force field [15]. The charges for CurE and ADP were assigned using Antechamber and the topologies file for Gromacs were prepared using Topolbuild, developed by Bruce D. Ray (personal communication). Amber ff12SB and GAFF force fields were used consistently for defining charges and topologies [15]. 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 the protein complex and its charge was neutralized using K^+ ions. During the MD simulations, we initially performed 50,000 steps of steepest descent minimization and 200 pico seconds (ps) position restrained dynamics to distribute water molecules throughout the system. Finally, we performed MD simulations of the whole system for 20 ns, using 0.002 ps time step. 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^{-5}. 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. The trajectory file obtained from MD simulations was used for calculating the free energy of binding, RMSD, hydrogen bonding etc. The visualisation studies and images were rendered using Chimera 1.8.

2.3. Energy evaluations

AutoDock 4.2 was used to calculate the energy of binding between F-actin and CurE and between F-actin and ADP at every 10 ps of the simulations. The coordinates of the centre atom of CurE, the 5th carbon of cyclopentane and ADP, the carbon atom next to the ribose sugar that connects the phosphates, were used as the grid centre and the same coordinates were retained for every conformation. *X*, *Y*, and *Z* grid dimensions of 62, 62 and 64 and 46, 50 and 48 were used for generating grid maps covering the binding site of ADP and CurE respectively. Estimated free energy of binding, electrostatic energy and internal energy of the ligand was extracted from the docking experiments to analyse the binding effects of CurE on F-actin and to see if there are any changes in the energy of ADP because of CurE binding to F-actin.

2.4. Motional correlations

DCC algorithm [16] from WORDOM [17] was used to calculate the cross-correlation analysis of the atomic fluctuations from the simulations. Twist angle between domain 1 and domain 2 were calculated using the same procedure as described before [18,19]. Residues Gly55 from subdomain 2 and Glu207 from subdomain 4 were used as reference points for domain 1 and domain 2 respectively for calculating the twist angle. Each MD snapshot was superimposed onto the monomeric ADP-Actin structure (PDB ID 1J6Z) [20] using do_multiprot [21]. The axis of rotation was determined between 1J6Z and the F-actin subunit (PDB ID 2ZWH) using DynDom [22]. The g_angle from GROMACS package was used to compute the dihedral angle distribution which was plotted to study the twist angle of different simulations.

2.5. QM/MM and DFT simulation experiments

The nucleophile sulphur of Cys257 attacks at the β-carbon of Michael Acceptor (MA) of CurE to produce an enol intermediate (Fig. 6A), a crucial intermediate of the Michael reaction. Usually, this intermediate collapses and the α-carbon gets protonated. The first step of the reaction where the nucleophile sulphur attacks the β-carbon, was derived using the QM/MM simulation experiment. In the next step, formation of an enol intermediate was explored using the DFT calculation. QM/MM study was carried out to derive the attach conformation of Cys257 against the Michael Acceptor of CurE using the coordinates derived from the last frame of the 20 ns MD simulation of CurE12 conformation. The QM part was simulated using the *ab initio* hybrid DFT with B3LYP functional and 6-31++G** basis sets and the MM part was simulated using the AMBER force field. The quantum partitions for the QM/MM simulations included the side chain of Cys257 and the double bonded β-carbon along with its hydrogen of the Michael Acceptor of CurE. QM/MM calculations were carried out for 500 QM interactions and 5000 MM interactions for 16 *ncycles*. The *ncycles* were interrupted at 16th cycle, because after this cycle there was no significant change in the QM/MM energy levels and conformations of the nucleophile and the CurE remained unaltered. DFT simulation was carried out to compute the overlapping molecular orbitals and the formation of the enol intermediate (covalent bond formation). The atomic coordinates of Cys257 and CurE from the last frame of the 20 ns MD simulation of CurE12 conformation, were used for DFT simulation. B3LYP functional and 6-31++G** basis sets was used to study the bond formation between Cys257 and CurE. All calculations were carried out using NWChem 6.5 program package [23]. The ground state wave functions were investigated by analysis of the frontier Molecular Orbitals (MOs) and the atomic contributions to MOs were calculated to analyse atoms involved in bond formation. Frontier MOs were calculated using the programs Jmol [24] and Chemissian 4.33 [25].

3. Results

3.1. Relevant mode of binding of CurE with F-actin was obtained using docking studies

Altogether, 24 different clusters obtained from the docking results were analyzed to select appropriate CurE binding conformations with F-actin for simulation studies. In the first 10 clusters summing up to 31 conformations CurE was bound at a distance greater than 7.5 Å from Cys257. Cluster 11 had 212 conformations, all of which had CurE binding closer to Cys257. The best