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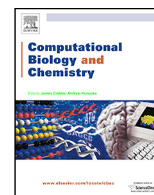

Research article

Extra precision docking, free energy calculation and molecular dynamics studies on glutamic acid derivatives as MurD inhibitors

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ABSTRACT

The binding modes of well known MurD inhibitors have been studied using molecular docking and molecular dynamics (MD) simulations. The docking results of inhibitors **1-30** revealed similar mode of interaction with *Escherichia coli*-MurD. Further, residues Thr36, Arg37, His183, Lys319, Lys348, Thr321, Ser415 and Phe422 are found to be important for inhibitors and *E. coli*-MurD interactions. Our docking procedure precisely predicted crystallographic bound inhibitor **7** as evident from root mean square deviation (0.96 Å). In addition inhibitors **2** and **3** have been successfully cross-docked within the MurD active site, which was pre-organized for the inhibitor **7**. Induced fit best docked poses of **2**, **3**, **7** and **15**/2Y1O complexes were subjected to 10 ns MD simulations to determine the stability of the predicted binding conformations. Induce fit derived docked complexes were found to be in a state of near equilibrium as evident by the low root mean square deviations between the starting complex structure and the energy minimized final average MD complex structures. The results of molecular docking and MD simulations described in this study will be useful for the development of new MurD inhibitors with high potency.

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1. Introduction

Peptidoglycan, the basic component of the bacterial cell wall is unique to prokaryotic cells. It is essential for the rigidity, flexibility and strength required for bacterial cells to grow and divide, as well as it protects individual bacterial cells against osmotic pressure (Vollmer et al., 2008); is thus an attractive target in antibacterial drug research (Barreteau et al., 2008; El Zoeiby et al., 2003). There has been increased interest in exploiting the early intracellular steps of peptidoglycan biosynthesis catalyzed by a group of cytoplasmic Mur enzymes (MurA-MurF) (Silver, 2003) to combat bacterial drug resistance. MurD is the second in the series of Mur ligases and catalyses the formation of peptide bond between cytoplasmic intermediate UDP-N-acetylmuramoyl-L-alanine (UMA) and D-glutamic acid (D-Glu). Its ubiquitous nature among the bacteria and its absence in mammals represents MurD as a promising target for the design of antibacterial agents. The high stereospecificity of MurD for D-glutamic acid (Pratviel-Sosa et al., 1994) and attempts to identify MurD inhibitors by using the transition-state hypothesis (Humljan et al., 2006, 2008) suggested D-Glu as an essential fragment of a potent inhibitor (Strancar et al., 2006). MurD from *Escherichia coli* is one of the best studied enzyme of the Mur ligase family. The binding site of this enzyme has three globular binding domains of interest for the design of MurD inhibitors (Bertrand et al., 1999, 2000; Tomasic et al., 2012). The N-terminal domain (UMA-binding site) accounts for the fixation of the UDP moiety of UMA. The central domain (ATP-binding pocket) appears to be well conserved in particular, throughout Mur enzyme family. This pocket is believed to be involved in the fixation of adenosine triphosphate (ATP), muramic acid and L-alanine moieties of UMA. The C-terminal domain also known as D-Glu binding site is responsible for the binding of the amino acid or dipeptide. The active site of MurD is located in the cleft between the central and C-terminal domain. UMA enters the cleft from the side closest to N-terminal domain whereas the ATP molecule from the opposite side. MurD bring together the UMA

Abbreviations: E. coli, Escherichia coli; Ecoul, Coulomb energy; Glu, Glutamic acid; IFD, Induced fit docking; MD, Molecular dynamics; meso-Dap, Meso-diaminopimelic acid; MurD, UDP-N-acetylmuramoyl-L-alanine D-glutamate ligase; ESP, Electrostatic electrostatic potentials; MM-GBSA, Molecular Mechanics-Generalized Born Surface Area; PSA, Polar Surface Area; PDB, Protein Data Bank; rGyr, Radius of Gyraion; RMSD, Root mean square deviation; SASA, Solvent accessible surface area; UDP, Uridine diphosphate; UMA, UDP-N-acetylmuramoyl-L-alanine; UMP, Uridine monophosphate; vdW, vanderWaals.

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and ATP and properly orient them for the formation of an acyl-phosphate intermediate. It further orients D-glutamine for the nucleophilic attack, and stabilizes the tetrahedral intermediate and accelerates the catalysis. The structural characteristics described above suggest that the amino acids that interact with the UMA and ATP could be exploited to improve the binding affinity of MurD inhibitors.

Residues present in the MurD active site of different bacterial species showed high homology and are well conserved (Bertrand et al., 1999, 2000). Several co-crystallized inhibitors with MurD (Bertrand et al., 1999, 2000, 1997; Tomasic et al., 2012) provided structural basis for the inhibitor improvement and optimization. Several attempts have been made to design potent inhibitors of MurD (El Zoeiby et al., 2003; Strancar et al., 2006; Umamaheswari et al., 2010; Frlan et al., 2008) but unfortunately, most of the MuD inhibitors designed and synthesized to date failed to show potent antibacterial activity. Structure-based design and structural modifications of thiazolidin-4-one based inhibitors (Perdih et al., 2009a) resulted in significant improvement of inhibitory activity against *E. coli* MurD (Tomasic et al., 2012). In another approach, second generation naphthalene-N-sulfonyl-D-glutamic acid derivatives were synthesized by substitution of the flexible D-Glu with different rigid fragments (Sosic et al., 2011). Further, virtual screening campaign based on the available MurD crystal structures co-crystallized with N-sulfonyl glutamic acid inhibitors (Kotnic et al., 2007; Tomasic et al., 2012 Tomasic et al., 2012) resulted in the discovery of glutamic acid surrogates benzene-1,3-dicarboxylic acid derivatives (Perdih et al., 2009b, 2014; Simcic et al., 2012). These compounds exhibited significant inhibitory activity against both MurD and MurE enzymes. Moreover, some of these conformationally rigid D-Glu mimetics showed activity against the whole cascade of Mur ligases (MurD-MurF) (Perdih et al., 2014). In recent years, computational methods were also utilized successfully (Samal et al., 2015; Simcic et al., 2014) for the development of MurD inhibitors. Based on the above facts, we report here molecular docking, binding free-energy calculation and molecular dynamics (MD) simulation studies of selected conformationally flexible D and L- glutamic acid-based inhibitors (**1-11**) and rigid analogs of D-glutamic acid (**12-30**) (Tomasic et al., 2011, 2012; Sosic et al., 2011; Humljan et al., 2008; Zidar et al., 2010, 2011; Kotnik et al., 2007; Perdih et al., 2009, 2014, 2015). Inhibitors were selected based on their wide range biological activity and structural diversity to analyze the binding modes of both conformationally flexible and rigid analogs of D-glutamic acid to explore the differences in potencies of these inhibitors. The role of hydrogen bonding of these inhibitors with key sites of the MurD enzyme is examined in detail. Molecular Mechanics-Generalized Born/Surface Area (MM-GBSA) analysis was carried out to calculate the binding free energies of proteins with inhibitors and MD simulations were performed to investigate the stability and dynamical changes of predicted binding conformations.

2. Computational details

2.1. Docking study

A data set comprising thirty *E. coli*-MurD inhibitors (**1-30**) was taken from literature (Tomasic et al., 2012, 2011; Sosic et al., 2011; Humljan et al., 2008; Zidar et al., 2011, 2010; Kotnik et al., 2007). The structure of these inhibitors and their biological activity (IC_{50} value) is shown in supplementary Table 1. The 3D structures of ligands were generated using the builder panel in Maestro 10.2 and subsequently optimized using the LigPrep module (v3.4, Schrödinger 2015-2). The OPLS_2005 force field (Shivakumar et al., 2010) was used for optimization to produce the low-energy conformers of each ligand. The X-ray crystal structure of *E. coli*-MurD in complex with the inhibitor **7** (PDB ID: 2Y1O) (Tomasic et al., 2012) was selected because of its high resolution (1.49 Å) and inhibitory activity (IC_{50}: 8.2 μM). It was prepared using the protein preparation wizard (Epik v3.2, Schrödinger suite 2015-2) (Sastry et al., 2013). Prior to protein optimization water molecules with less than three hydrogen bonds were removed from the crystal structure. Further, hydrogen bonds (corresponding to pH 7.0) and missing side chain atoms were added and breaks present in the protein structure were repaired with Prime (v4.0, Schrödinger 2015-2) (Jacobson et al., 2004). This was followed by the energy minimization of protein under OPLS_2005 force field (Shivakumar et al., 2010) with convergence of heavy atoms to a root mean square deviation (RMSD) of 0.3 Å. The Ramachandran plot (Ramachandran et al., 1963) (Supplementary Fig. 1) was generated for this protein (PDB ID: 2Y1O) under protein preparation wizard (Epik v3.2, Schrödinger suite 2015-2). It showed 97.8% of the residues in the most favored regions and none of the non-glycine residues in disallowed regions. A 10 Å 3D-grid box was generated defining the co-crystallized ligand as centroid of the active site. The low energy conformations of all inhibitors were docked into the catalytic pocket of the study model using extra precision (XP) mode (Glide v6.7, Schrödinger 2015-2) (Friesner et al., 2006) which incorporates water desolvation energy and protein-ligand structural motifs terms into the binding free energy scoring function with enhanced ability of Glide to pick out known active compounds from a random ligand database. Based on Glide score, Glide energy and Glide model energy (Fig. 1 also Supplementary Table 2 and Fig. 2), the best docked structure of each ligand was selected. Further, we generated the map of hydrophobic and hydrophilic fields for inhibitors **2**, **3**, 5 and **7** (Supplementary Fig. 3).

2.2. Binding free energy calculation using prime/MM-GBSA approach

To calculate the free energy of binding for inhibitors **1-30** to the MurD structure model (PDB ID: 2Y1O), the Prime/Molecular Mechanics-Generalized Born Surface Area (MM-GBSA) (v4.0, Schrödinger 2015-2) (Jacobson et al., 2004) approach was used. The docked poses of all inhibitors in the catalytic pocket of study model were minimized using the local optimization feature in Prime and the simulation was performed. The energies of complex were calculated with the OPLS_2005 force field (Shivakumar et al., 2010) and Generalized-Born/Surface Area (MM-GBSA) continuum solvent (VSGB 2) model (Li et al., 2011) (Table 1).

2.3. Induced-fit docking

The induced-fit docking (IFD) with extended sampling protocol was performed for inhibitors **2**, **3** and high active inhibitors **7** and **15**. It uses the docking program Glide (v6.7 Schrödinger 2015-2) to account for the ligand flexibility and the refinement module in Prime (v4.0, Schrödinger 2015-2) (Jacobson et al., 2004) to account for the receptor flexibility. IFD scores that accounts for both the protein-ligand interaction energy and the total energy of the system was calculated and used to rank the IFD poses. The best pose **2**, **3**, **7** and **15**/2Y1O complexes were selected to run the molecular dynamics.

2.4. Molecular dynamics (MD) stimulation

The molecular dynamics simulations (Guo et al., 2010) were performed for the docked complexes of **2**, **3**, **7** and **15** with MurD protein (PDB-ID: 2Y1O) using the OPLS_2005 force field (Shivakumar et al., 2010). All systems were solvated in orthorhombic boxes with explicit TIP3P (Jorgensen et al., 1983) water within the Desmond molecular dynamics system (v4.2). Each system was neutralized by adding counter ions. Smooth Particle Mesh Ewald

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