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Molecular dynamics simulations studies and free energy analysis on inhibitors of MDM2–p53 interaction

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ABSTRACT

The oncoprotein MDM2 (murine double minute 2) negatively regulates the activity and stability of tumor suppressor p53. Inactivation of the MDM2–p53 interaction by potent inhibitors offers new possibilities for anticancer therapy. Molecular dynamics (MD) simulations were performed on three inhibitors–MDM2 complexes to investigate the stability and structural transitions. Simulations show that the backbone of MDM2 maintains stable during the whole time. However, slightly structural changes of inhibitors and MDM2 are observed. Furthermore, the molecular mechanics generalized Born surface area (MM-GBSA) approach was introduced to analyze the interactions between inhibitors and MDM2. The results show that binding of inhibitor pDIQ to MDM2 is significantly stronger than that of pMI and pDI to MDM2. The side chains of residues have more contribution than backbone of residues in energy decomposition. The structure–affinity analyses show that L54, I61, M62, Y67, Q72, H73 and V93 produce important interaction energy with inhibitors. The residue W/Y22′ is also very important to the interaction between the inhibitors and MDM2. The three-dimensional structures at different times indicate that the mobility of Y100 influences on the binding of inhibitors to MDM2, and its change has important role in conformations of inhibitors and MDM2.

1. Introduction

In tumors, p53 protein is a potent inducer of cell cycle arrest, DNA repair, cellular senescence, innate immunity and apoptosis [1–3]. The E3 ubiquitin ligase MDM2 is one of the principal p53 modulators. It binds directly to the p53 transactivation domain and inhibits p53-dependent transcription [2,4]. Thus, reactivation of p53 by the inhibition of its binding to MDM2 is regarded as an effective and confirmed approach in cancer therapy [5]. Many reports have demonstrated the relevance between MDM2 inhibition and growth inhibition of cancer cells [4,6,7].

The side chains of hydrophobic residues F19′, W23′ and L26′ are responsible for the interaction of p53 with MDM2 [8–10]. The binding of p53 to MDM2 is directly disrupted by these residues and it may be an attractive pathway of targeted anticancer therapy [11–13]. Many drug candidates, such as small-molecule inhibitors, peptides, and peptide-analog are designed to target the interaction between p53 and MDM2. And the design novel potent inhibitors have become the current goal for cancer therapy development [14,15].

Recently, two peptide inhibitors pDI (LTFEHYWAQLTS) and pMI (TSFAEYWNLLSP) was identified using phage display [2,16–18]. Using pDI and pMI for comparison, a quadruple mutant peptide (pDIQ) was reported as the most potent inhibitor against MDM2 [19]. And the IC_{50} values determined by ELISA are pDIQ (8 nm), pMI (20 nm) and pDI (44 nm), respectively [19]. Three residues F19′, W23′ and L26′ of the inhibitors are critical residues for binding to MDM2. The dynamics, flexibility and conformational changes of three complexes have not been detailed discussed.

Molecular dynamics (MD) simulations are a powerful tool to complement experimental results with detailed dynamics behavior of biomolecules [20,21]. Binding free energy calculations and analysis have been proven to be powerful and valuable tools for understanding mechanisms of inhibitors to proteins [22]. MM-GBSA method has been proposed to be one of effective methods to calculate the binding free energies of inhibitors to proteins [23–25]. It has been successfully used to explain protein–protein and protein–inhibitor interactions [26–32]. In this work, molecular dynamics simulations combined with MM-GBSA method was applied to study the binding free energies of three inhibitors to MDM2. In addition, the energy decomposition analysis was carried out with the MM-GBSA approach. The detailed van der Waals, electrostatic, polar solvation, and nonpolar solvation energies between these inhibitors and individual MDM2 residues were calculated using per-residue-based decomposition method [33]. These results provide the difference in binding modes of the three inhibitors and

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reveal the main energy contribution of inhibitors binding to MDM2. In addition, the effects of the inhibitors and residue Y100 on the dynamics of MDM2 were analyzed. We suggest that these results can provide useful insights into the mechanisms of inhibition of the p53–MDM2 interaction.

2. Methods

2.1. System setups

The crystal structures of MDM2 complex with three peptide inhibitors (pDIQ, pMI and pDI) were obtained from the protein data bank (PDB) [34]. The PDB entries are 3JZS, 3EQS, and 3G03 [17–19]. These three structures were used for the starting model of MD simulations. All missing hydrogen atoms in MDM2 were added with the leap module in AMBER 11 package [35].

The ff99SB force field was applied to produce the parameters for the three models. An appropriate number of Cl^- counter ions were added to neutralize the charges of the systems. Finally, the whole system was solvated in an octahedral periodic box of TIP3P water molecules, and the distance between the edges of the water box and the closest atom of the solutes was at least 9.0 Å.

2.2. Molecular dynamics simulations

Energy minimizations and MD simulations were performed for each system using the SANDER module of AMBER 11 package. First, the water molecules and counter ions were minimized by positional restraints of 100 kcal/(mol Å^2) on the protein and inhibitor atoms to remove the bad contacts. Second, the entire system was minimized without any restraint. Each step was consisted of a 4000-step steepest descent and a 4000-step conjugate gradient minimization. After minimization, the system was gradually heated from 0 to 300 K in 200 ps with a position restraint of 10 kcal/(mol Å^2) in the C_α atoms of the complex. This followed by constant temperature equilibration at 300 K for another 200 ps. Finally, 50 ns MD simulations of each system at 1 atm and 300 K were carried out in an isothermal isobaric ensemble (NPT) with periodic boundary conditions. During the simulation, the SHAKE method was applied to constraint the covalent bonds involving hydrogen atoms [36,37]. An integration step of 2 fs was set for the MD simulations and the Particle Mesh Ewald (PME) method was used for calculating the long-range electrostatic interactions [38,39]. The cutoff distances for the long-range electrostatic and van de Waals energy interaction were set to 12 Å.

2.3. MM-GBSA calculation

For each complex, 1000 snapshots were extracted from the last 10 ns along the MD trajectory at an interval of 10 ps. The MM-GBSA method and nmod module, which implemented in Amber 11, were performed to compute the binding free energies of the three complex systems. In this method, the binding energy (ΔG) can be represented as:

$$\Delta G_{\text{bind}} = G_{\text{complex}} - G_{\text{protein}} - G_{\text{inhibitor}} \quad (1)$$

each free energy term in Eq. (1) is computed as a sum of gas phase molecular mechcanical energy (E_{gas}), the solvation free energy (E_{sol}), and the entropy term ($-T\Delta S$), using Eq. (2):

$$\Delta G_{\text{bind}} = E_{\text{gas}} + G_{\text{sol}} - T\Delta S \quad (2)$$

E_{gas} can be further divided into two parts:

$$E_{\text{gas}} = E_{\text{ele}} + E_{\text{vdW}} \quad (3)$$

where E_{ele} and E_{vdW} are described as the electrostatic interaction and van der Waals energy in the gas phase, respectively. The solvation free energy is expressed as:

$$G_{\text{sol}} = G_{\text{gb}} + G_{\text{nonp}} \quad (4)$$

where G_{gb} and G_{nonp} are the polar and non-polar contributions to solvation free energy. The polar component was computed using the GBSA program. The dielectric constants were set to 1 and 80 for the solute and surrounding solvent respectively in our calculations. The non-polar contribution was defined by the equation:

$$\Delta G_{\text{nonp}} = \gamma \text{SASA} + \beta \quad (5)$$

where SASA is the solvent accessible surface area estimated with a probe radius of 1.4 Å. γ and β are empirical constants and were set as 0.0072 kcal/(mol Å^2) and 0 kcal/mol for GB method. The conformational entropy change upon inhibitor binding ($-T\Delta S$), was obtained from the sum of the translational, rotational, and vibrational components, with the Nmode module of Amber 11.

2.4. Inhibitor–residues interaction decomposition

The interaction energies were further decomposed into contributions from protein and inhibitor residue pairs, which can only use the MM-GBSA method. The binding energy of each residue pair consists of three terms:

$$\Delta G_{\text{inhibiotr-residue}} = E_{\text{ele}} + E_{\text{vdW}} + G_{\text{gb}} + G_{\text{surf}} \quad (7)$$

where E_{ele} and E_{vdW} are described as non-bonded electrostatic interaction and van der Waals energy between the inhibitor and each MDM2 residue in the gas phase, respectively. And G_{gb} and G_{surf} are the polar and non-polar contributions for the inhibitor–residue interaction.

3. Results and discussion

3.1. Stability during MD simulations

To evaluate the reliable stability of the MD trajectories and the differences in the stabilities of MD simulations, the RMSD values of C_α atoms of MDM2 with respect to the starting structure over the 50 ns simulations are monitored. As shown in Fig. 1, the three complexes have reached the equilibrium and the RMSD values of 3JZS, 3EQS and 3G03 are ~1.20 Å after the 30 ns simulations, indicating good agreement with the X-ray crystal structures. And the deviations are under 0.12 Å in all three MD simulations. These results showed that the trajectories of MD simulations we used for post analyses of the three complexes are reliable.

To quantitatively measure the mean backbone mobility for each residue, the root mean square fluctuations (RMSF) values of MDM2 C_α relative the starting structure over the 50 ns simulations were calculated, as shown in Fig. 2. The results indicate that the mobility of the loops L2, L5 and the helixes $\alpha1'$, $\alpha2$, $\alpha2'$ domains are more obvious in the 3G03 structure than in 3JZS and 3EQS structures.

3.2. Binding free energies calculations

To further evaluate the difference in the binding modes of inhibitors to MDM2 and obtain detailed insights into the effect of each component contributed to the inhibitor–protein binding, the binding free energies of protein–inhibitor complexes are examined using MM-GBSA methods. The calculated results and experimental data are summarized in Table 1. The binding free energies of inhibitors to MDM2 are 3JZS (−21.03 kcal/mol), 3EQS (−14.62 kcal/mol) and 3G03 (−13.57 kcal/mol), respectively. These