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Insight into the interaction mechanism of inhibitors P4 and WK23 with MDM2 based on molecular dynamics simulation and different free energy methods

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ABSTRACT

The p53–MDM2 interaction has been an important target for the designs of anticancer drugs. In this work, molecular dynamics (MD) simulations combined with molecular mechanics generalized Born surface area (MM-GBSA) and solvated interaction energy (SIE) methods were applied to calculate binding free energies of MDM2 with the peptide inhibitor P4 and non-peptide inhibitor WK23. The binding free energies predicted by two different free energy methods agree well with the experimental values. The results suggest that van der Waals interaction is the main force of inhibitor bindings to MDM2. Dynamics analysis and the inhibitor–residue interactions were also performed. The results show that the CH–CH, CH–π and π–π interactions drive the bindings of inhibitors to MDM2 and the peptide inhibitor P4 can produce strong interactions with more residues of MDM2 than WK23. We expect that this study can provide important helps for the designs of potent inhibitors targeting the p53–MDM2 interactions.

1. Introduction

The tumor suppressor p53 is responsible for regulating cell cycle arrest, apoptosis and DNA repair upon cellular stress [1,2]. Active p53 protein can efficiently inhibit the development of tumor. However, the activity of p53 can be inhibited by MDM2, a protein which can inhibit p53 abilities to bind to DNA and activate transcription by interacting with p53 [3,4]. In fact, almost 50% of all human cancers in the world are owed to the invalidation of p53 function caused by deletions or mutations in the DNA-binding domain of p53 [5]. Thus, the p53–MDM2 interaction becomes an attractive molecular target for cancer therapy.

P53 can interact with MDM2 by inserting its hydrophobic face (Phe19′, Trp23′ and Leu26′) into a deep groove in MDM2, and direct disruption of the p53–MDM2 interaction has become an attractive target for anticancer therapy [5]. Currently, many peptide inhibitors that mimic the p53–MDM2 interaction have been reported in the previous scientific researches [6–11]. Although these peptide inhibitors possess high affinity for MDM2, they display only modest potency in a cellular context, presumably because of their poor membrane permeabilities [12,13]. Several classes of non-peptide small molecule compounds reported to date, such as the cis-imidazolines (nutlins), isoindolinone and spiro-oxindoles (MI-63), have good membrane permeabilities, however, their binding abilities to MDM2 are poorer than those of the peptide inhibitors [14–18]. Thus, it is essential to study the interaction mechanism of peptide and non-peptide inhibitors with MDM2 at atomic levels for the designs of potent drugs targeting the p53–MDM2 interaction.

Molecular simulations based on computer technology have been proven to be powerful and valuable tools for understanding the binding mechanisms of inhibitors to proteins [19–23]. Many simulation studies have been performed on the p53–MDM2 interaction. Ding et al. studied residue-specific interactions between p53 and MDM2 by using quantum mechanics calculations, and their results suggested that van der Waals interactions control the p53–MDM2 binding [24]. The calculations of binding free energies based on MD simulations were carried out by other groups and also obtained similar conclusions to Ding et al. [24–28]. Computational alanine-scanning mutagenesis performed by several groups showed that single point mutations of four key positions (Phe19, Leu22, Trp23 and Leu26) of p53 resulted in a significant decrease in binding free energies [29–31]. In addition, the conformational change of MDM2 induced by ligand binding is also very important to guide rational drug design. Chen et al. and Espinoza Fonseca et al. applied MD simulation to probe the conformation changes of MDM2, and their results revealed that the most flexible

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region of MDM2 was the p53-binding cleft [32,33]. Thus, because of the importance of the p53–MDM2 interaction, the understanding of the binding modes of peptide and non-peptide inhibitors to MDM2 is still essential for the designs of potent inhibitors targeting the p53–MDM2 interaction.

In order to probe the binding modes of two kinds of inhibitors to MDM2, the peptide inhibitor P4 and non-peptide inhibitor WK23 were selected. P4 is a peptide inhibitor (LTFEHYWAQLTS) designed by Czarna et al. [10], and structurally shares three key residues (Phe19, Trp23 and Leu26) with p53 (Fig. 1A and B). This inhibitor shows strong inhibition potency (*Ki* value of 4 nM) on the p53–MDM2 interaction [10]. WK23 is a non-peptide inhibitor designed by Popowicz et al., and structurally mimics the interaction of p53 with MDM2 (Fig. 1C and D). It can produce potent inhibiting effects with K*i* value of about 916 nM [34]. In this work, two different free energy methods, molecular mechanics generalized Born surface area (MM-GBSA) [35–37] and solvated interaction energy (SIE) [38,39], were applied to calculate the binding free energies of P4 and WK23 to MDM2 and probe their binding mode differences to MDM2. This study helps to clarify the molecular basis of the inhibition of the p53–MDM2 interaction. We also expect that this study will provide significant contributions to the designs of anticancer drugs targeting the p53–MDM2 interaction.

2. Theory and method

2.1. System initialization

Two X-ray-structures selected from protein data bank (PDB) were studied in this work: 3G03 for the P4–MDM2 complex [10] and 3LBK for the WK23–MDM2 complex [34], while the structure of free MDM2 is extracted from the P4–MDM2 complex (3G03). Due to the difference in the lengths of the protein sequences, only residues 26–108 were used for this study. Furthermore, the amino terminus Thr26 and the carbonyl terminus Val108 were capped by an acetyl group (ACE) and an N-methyl group (NME), respectively. All missing hydrogen atoms in MDM2 crystal structure were added by using the Leap module in Amber12 software package [40]. All crystal water molecules in the inhibitor–MDM2 complex were kept in the starting model. The force field ff03 was applied to produce the force field parameters of the protein and crystal water molecules [41]. The structure of WK23 was minimized at the semi-empirical AM1 level and AM1-BCC charges were assigned to WK23 by running Antechamber program in Amber 12. The general Amber force field (GAFF) was adopted to obtain the force field parameters of WK23 [42]. An appropriate number of chloride counterions were placed around two MDM2–inhibitor complexes to neutralize the systems. Finally, each 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 12.0 Å.

2.2. MD simulations

Energy minimization and MD simulation were carried out for each system by using the Sander module of the Amber 12 program. Before MD simulations, each system was subject to energy minimization in two stages to remove bad contacts between the complex and solvent molecules. Firstly, the water molecules and counterions were minimized by freezing the solute with a harmonic constraint of a strength of 100 kcal mol^{-1} $Å^{-2}$. Secondly, each system was minimized without restriction. Each stage was performed by using the steepest descent minimization of 2500 steps followed by a conjugate gradient minimization of 2500 steps. Then, the system was heated from 0 to 300 K in 200 ps and equilibrated at 300 K for another 200 ps. Finally, 20-ns MD simulations were run on each system at 1 atm and 300 K. In order to use longer time step, the SHAKE method was applied to constraint the covalent bonds related to hydrogen atoms [43]. The Particle Mesh Ewald (PME) method was used for treating the long-range electrostatic interactions [44,45]. The cutoff distances for the long-range electrostatic and van der Waals energy terms were set to 12.0 Å.

2.3. MM-GBSA method

Binding free energies of P4 and WK23 to MDM2 were calculated by using MM-GBSA program in Amber 12 [35,36,46]. For each

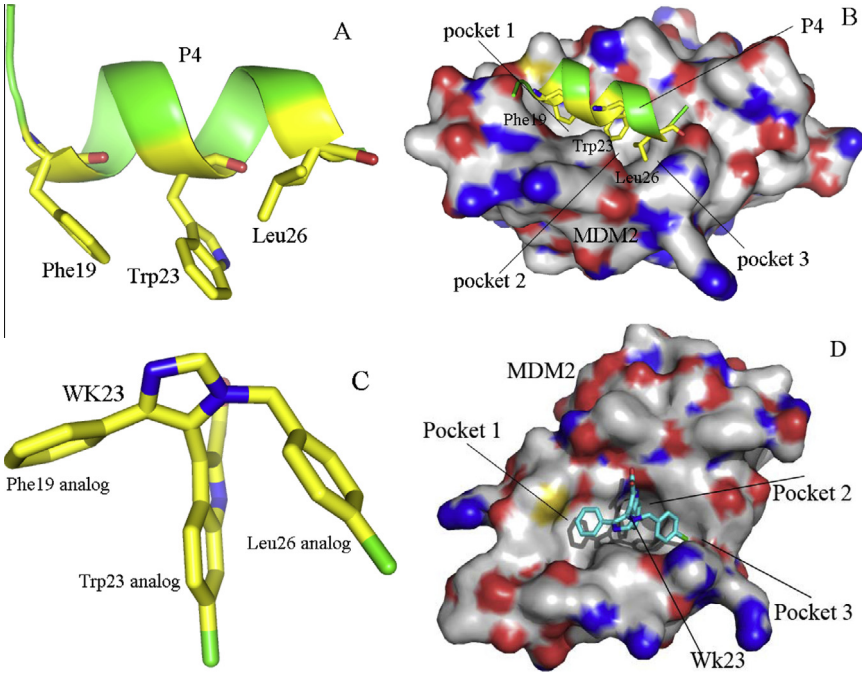

Fig. 1. Structures of inhibitors and inhibitor–MDM2 complexes: A for P4, B for the P4–MDM2 complex, C for WK23 and D for the WK23–MDM2 complex.