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Binding mechanism of CDK5 with roscovitine derivatives based on molecular dynamics simulations and MM/PBSA methods

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ABSTRACT

Roscovitine derivatives are potent inhibitors of cyclin-dependent kinase 5 (CDK5), but they exhibit different activities, which has not been understood clearly up to now. On the other hand, the task of drug design is difficult because of the fuzzy binding mechanism. In this context, the methods of molecular docking, molecular dynamics (MD) simulation, and binding free energy analysis are applied to investigate and reveal the detailed binding mechanism of four roscovitine derivatives with CDK5. The electrostatic and van der Waals interactions of the four inhibitors with CDK5 are analyzed and discussed. The calculated binding free energies in terms of MM-PBSA method are consistent with experimental ranking of inhibitor effectiveness for the four inhibitors. The hydrogen bonds of the inhibitors with Cys83 and Lys33 can stabilize the inhibitors in binding sites. The van der Waals interactions, especially the pivotal contacts with Ile10 and Leu133 have larger contributions to the binding free energy and play critical roles in distinguishing the variant bioactivity of four inhibitors. In terms of binding mechanism of the four inhibitors with CDK5 and energy contribution of fragments of each inhibitor, two new CDK5 inhibitors are designed and have stronger inhibitory potency.

1. Introduction

Cyclin-dependent kinases (CDKs) are a family of heterodimeric serine/threonine protein kinases, each of which includes catalytic CDK subunit and an activating cyclin subunit. They can control the progression of the cell cycle [1]. The cyclin-dependent kinase 5 (CDK5), discovered in 1992 [2–4], is involved in many neuronal diseases, such as traumatic brain injury, stroke, cell migration, Alzheimer diseases (ADs), and so on [5]. In fact, similar to other CDKs, separate CDK5 does not exhibit activity, and can be activated by combining one or two cyclin subunits, p35 or p39 in this system [2]. Some researchers suggested that p35 is an irreplaceable activator comparing with p39 [6]. p25, a proteolytic segment, is truncated from p35, contains the C-terminal portion of p35, and has high concentrations in AD patients [7]. The interaction of CDK5 and p25 can extend to the T-loop of non-phosphorylated CDK5, that is, if the complex of CDK5/p25 forms (higher activity than CDK5/p35 [8]), CDK5/p25 without phosphorylation would be immersed in absolutely active state [9]. Therefore, the CDK5/p25 complex has become an attractive pharmacological target. The design of novel CDK5/p25 inhibitors attracts much interest in recent years [1,10–19].

Recently, many researchers designed and synthesized several kinds of inhibitors of CDK5/p25 including purine [13], bisindoles [12,14], aloisines [15], quinolin-one [16], amino-thiazoles [17], paullones [18,19] and other typical inhibitors [1]. Theoretically, Haq et al. [20] applied the molecular docking and 3D-QSAR modeling (CoMFA and CoMSIA) to reveal the important interactions between the receptor active site residues and compound's functional groups and to understand the binding orientation. Cavalli et al. [21] investigated protein–ligand interaction in CDK5 in terms of steered molecular dynamics simulations. Ji et al. [22] studied the selectivity of paullones inhibiting GSK-3 rather than CDK5 based on molecular dynamics simulations and free-energy calculations, revealing that paullones could be used as potent selective inhibitors. One inhibitor of purines, (R)-roscovitine [2-(*R*)-(1-ethyl-2-hydroxy-ethylamino)-6-benzylamino-9-isopropyl-purine] containing an asymmetric carbon can inhibit CDK5/p25 and other CDKs [12,23,24] and is a promising CDK inhibitor currently in clinical trials (CDK5/p25,IC50 = 0.2 μM) [23]. It has been found that the roscovitine could make an unusual collapsed conformation of the glycine-rich loop (an important site of CDK regulation) more stable [12]. The results obtained by Tan et al. [24] demonstrated that it is possible to obtain more efficient roscovitine-like inhibitors through

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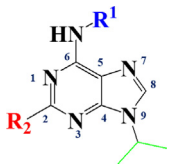
Table 1
Structures and in vitro activity of roscovitine derivative inhibitors of CDK5.



Inhibitors[a]	R^1	R^2	IC50/(μM) [5]
A1	−4-pyridine	(*R*)-1-Hydroxy-but-2-ylamino	0.017
A2	−benzyl	(*R*)-1-Hydroxy-but-2-ylamino	0.2
A3	−2-pyridine	(*R*)-1-Hydroxy-but-2-ylamino	2.3
A4	−4-pyridine	Cl	7.4

[a] Each CDK5 inhibitor is divided into four fragments, which are displayed by different colors, that is, black represents fragment A (9*H*-Purin-6-amine group); blue, fragment B (R^1 group); red, fragment C (R^2 group); and green, fragment D (isopropyl group).

modifying the structure of roscovitine to improve the electrostatic field, leading to more complementary to the protein binding site. In aspect of the drug design, Zhu et al. [25] suggest that inhibitors with larger rotatable side-chain are unfavorable for binding with CDK5 by comparing with inhibition mechanism of three different inhibitors ((*R*)-roscovitine (RRC), aloisine-A (ALH) and indirubin-3′-oxime (IXM)) with CDK5.

In spite of the above experimental and theoretical studies, so far, the detailed binding mechanism of roscovitine derivatives (see Table 1) with CDK5/p25 (simplified as CDK5 hereunder) has not been clearly understood. Molecular dynamics (MD) simulation [21,22,24,25] can be used to solve the above problem in terms of experimental results. Revealing the reason why the four roscovitine derivatives (see Table 1) with CDK5 have different inhibitory potency can help us gain some insights into the binding modes and binding mechanism of them with CDK5, and provide new ideas for the design of more promising CDK5 inhibitors. Herein, we apply molecular docking, molecular dynamics (MD) simulation, and binding free energy calculation to investigate and reveal the binding mechanism between four roscovitine derivatives and CDK5. The main findings can be described as follows. First, the computed binding free energies are in agreement with experimental inhibitory patency of four inhibitors. Second, the hydrogen bonds of inhibitors with Cys83 and Lys33 are favorable to binding affinity of CDK5 with inhibitors. Third, the van der Waals energies dominate the binding free energies of CDK5/inhibitor complexes, especially the key contacts with Ile10 and Leu133 play important roles in distinguishing the variant bioactivity of four inhibitors. Fourth, based on the binding mechanism of the four inhibitors with CDK5 and energy contribution of fragments of each inhibitor, the two newly designed CDK5 inhibitors in current work have stronger inhibitory potency.

2. Computational details

2.1. Initial structure preparation

The starting geometry of CDK5 for molecular docking is got from the X-ray structure 1UNL of dimeric CDK5 (chains A and B) complex with the activator p25 (chains D and E) and with one inhibitor RRC (called A2 in this paper) in the RCSB Protein Data Bank (PDB: 1UNL, resolution 2.20 Å) [12]. In this work, the four roscovitine derivatives are taken as CDK5 inhibitors constructed by 3D graphical software as shown in Table 1. Geometric optimization and the electrostatic potential calculations are performed at the B3LYP/6-31G(d) level [26] using Gaussian 09 software [27].

2.2. Docking calculations

The crystal structure of CDK5/A2 can be obtained from the RCSB Protein Data Bank (PDB: 1UNL, resolution 2.20 Å) [12]. In order to examine the reliability of docking method of Autodock4.0 software [28], first, the inhibitor RRC (A2) is extracted from the crystal structure CDK5/A2 (PDB ID:1UNL), then it is docked into the ATP binding site of CDK5 [13,24] by Autodock4.0. The corresponding structure is similar to its crystal structure, suggesting the docking method of Autodock4.0 is relatively reliable. Therefore, other inhibitors are docked to CDK5, respectively, using Autodock4.0. The detailed docking process is shown as follows. The other three inhibitors are also docked to the ATP binding site of CDK5, respectively, based on Autodock4.0 program using the Lamarckian genetic algorithm [28]. Before docking, polar hydrogen atoms are added to CDK5 and Kollman all-atom charges are assigned to this enzyme. During each molecular docking process, CDK5 is set to be rigid, while automatic bond settings are used, allowing the torsion angles of all acyclic, rotatable bonds in the ligand to vary except for amide bonds. The grid maps are centered on the ATP site of CDK5, and the three dimensions of the grid are $90 \times 90 \times 90$ points with a 0.375 Å spacing value, therefore, there is sufficient space to include most of the protein and the active sites. For each of the 200 independent genetic algorithm runs, a maximum 25,000,000 energy evaluations is performed using the default operator weights and a population size of 300. The other docking parameters are set to the default values. Here, the root-mean-square deviation (RMSD) fluctuations of backbone heavy atoms, whose values are all less than 2.0 Å, are clustered together. After clustering analysis, the structures with lower mean binding free energy and the larger number of conformations are chosen as the preferred docking conformations.

2.3. Molecular dynamics simulations

The LEAP module of the Amber10 package [29] is used for the addition of all missing hydrogen atoms of the protein and ligands. The restrained electrostatic potential (RESP) method [26,30,31] is used to determine its partial atomic charges at the B3LYP/6-31G(d) level. The standard AMBER force field (ff03) [32] and the general AMBER force field (gaff) [33] are applied to describe the interactions of protein and ligand, respectively. Each system is immersed into an octahedron periodic box with at least 10 Å distance around the complex, containing approximately 7600 TIP3P [34] water molecules, and the minimum distance between each protein and box walls is set as 10 Å. The appropriate numbers of sodium ions are included to maintain each system electro-neutrality. Then, all molecular dynamics simulations are carried out using Amber10 package. First, each system is minimized by two steps: (1) applying harmonic restraints to all protein atoms and the inhibitor and (2) allowing all atoms to move freely in turn. Each energy minimization is performed by the steepest descent method for the first 2000 steps and the conjugate gradient method for the subsequent 2000 steps. Then, a position restrained dynamics simulation is conducted on each system. CDK5, each inhibitor, and water molecules are coupled separately to a temperature bath of 300 K with a coupling time of 0.1 ps. Ultimately, a 20 ns MD simulation is carried out on each system. During MD simulation processes, the Particle Mesh Ewald (PME) [35] is employed to deal with the long-range electrostatic interactions, and all covalent bonds containing hydrogen atoms are constrained using the SHAKE algorithm [36]. The coordinates are saved every 1 ps for the subsequent binding free energy calculation and the energy decomposition analysis.