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Computational determination of binding structures and free energies of glucose 6-phosphate dehydrogenase with novel steroid inhibitors

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ABSTRACT

Glucose 6-phosphate dehydrogenase (G6PD), the first and the rate-limiting enzyme in the pentose phosphate pathway (PPP), catalyzes the oxidation of G6P to 6-phosphogluconolactone and the reduction of $NADP^+$ to NADPH. Its key role in cancer promotes the development of a potent and selective inhibitor that might increase cancer cell death when combined with radiotherapy. In the present study, we investigated the detailed binding modes and binding free energies for G6PD interacting with a promising series of recently developed inhibitors, i.e., the steroid derivatives, by performing molecular docking, molecular dynamics (MD) simulations, and binding free energy calculations. The docking indicates that the inhibitors occupy the binding sites of both G6P and $NADP^+$. The calculated binding free energies on the basis of the MD-simulated enzyme–inhibitor complexes are in good agreement with the experimental activity data for all of the examined inhibitors. The valuable insights into the detailed enzyme–inhibitor binding including the important intermolecular interactions, e.g., the hydrogen bond interaction and the hydrophobic interaction, have been provided. The computational results provide new insights into future rational design of more potent inhibitors of G6PD as a treatment for cancer.

1. Introduction

In recent years, the role of metabolism in the development and maintenance of cancer has been widely studied. Many metabolic reactions can both quickly generate adenosine triphosphate (ATP) and feed the requirement for new lipids and nucleotides, leading the unrestricted proliferation of tumors. The pentose phosphate pathway (PPP), conserved in humans, animals, plants and microorganisms, is an important pathway involved in the metabolite production. Through this pathway, the substrate, glucose 6-phosphate (G6P), is converted to ribulose 5-phosphate (Ru5-P), used for the synthesis of nucleotide and the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) is also generated. NADPH not only is involved in macromolecular biosynthesis as a cofactor for many enzymes, but also plays the important role in maintaining the activity of antioxidants, protecting cells against reactive oxygen species (ROS) generated in rapid proliferation of cells.

Glucose 6-phosphate dehydrogenase (G6PD, EC1.1.1.49) is the first and the rate-limiting enzyme in the PPP and catalyzes the oxidation of G6P to 6-phosphogluconolactone and the reduction of $NADP^+$ to NADPH. In the past years, the importance of G6PD in cancer has been highlighted. In normal cells, the expression of G6PD enzyme is tightly controlled [1]. However, G6PD is overexpressed in many tumors, resulting in a remarkable increase of the G6PD activity in a variety of swollen tumor tissues, including bladder cancer [2], renal cell carcinoma [3], ovarian cancer [4], fiber meat tumor [5], breast cancer [6], endometrial cancer [7], cervical cancer [8], prostate cancer [9,10], and lung cancer [11]. In contrast, tumor cells with low G6PD activity grow more slowly and exhibit enhanced apoptosis [12,13].

Due to the close relationship between G6PD with cancer, new anticancer drugs which can inhibit the activity of G6PD could be designed by through two different directions, including silencing the gene expression of G6PD to prevent the generation of G6PD [13,14] and discovering the effective compounds against the enzymatic activity of G6PD [15,16]. A competitive G6PD inhibitor, 6-aminonicotinamide (6AN), was used for chemotherapy of various cancers, but had severe side effects, including nerve damage and vitamin B deficiency [15]. Steroids including dehydroepiandrosterone (DHEA) and epiandrosterone (EA) were reported to uncompetitively inhibit G6PD activity in 1960 [17].

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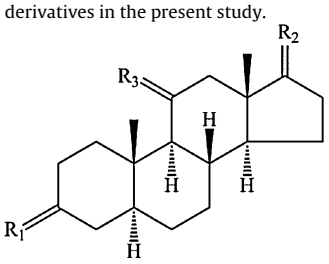
Table 1
Molecular structures and G6PD inhibitory activities of 6 representative steroid DHEA derivatives in the present study.



Compound	R_1	R_2	R_3	IC_{50}(μM)
1	α-H, β-OH	α-H, β-$COCH_2OH$	H_2	0.9
2	α-H, β-OH	α-OH, β-$COCH_2OH$	H_2	5.2
3	α-H, β-OH	α-OH, β-$COCH_3$	O	47
4	α-H, β-OH	α-H, β-CH_2COCH_3	H_2	>200
5	α-NH_2, β-H	O	H_2	>200
6	α-OH, β-H	O	H_2	>200

However, clinical trials with DHEA were unsuccessful because of the required high oral doses and the conversion of DHEA into the active androgens [18]. Some attempts have been performed to improve the activity through synthetic modification [19,20] and electrostatic potential map analysis [21], resulting in the discovery of 16α-bromo substituent of DHEA and EA with the remarkably increased inhibition activity. However, none has a remarkable selectivity for inhibiting G6PD among these reported molecules [15,16]. Recently, Hamilton et al. designed novel derivatives of the steroid DHEA with approximately 10-fold improved inhibition activity against G6PD [22].

In order to design more potential inhibitors, it is fundamental and necessary to reveal the detailed binding modes of G6PD with these inhibitors and understand the key interactions in the binding. The detailed analysis on the reported G6PD inhibitors indicated that G6PD inhibitory activity requires a β–alcohol group at R_1 position and a ketone group at R_2 position as shown in Table 1 [22]. In order to reveal the important role of these substituent groups on the binding, six steroid DHEA derivatives which meet this structural requirement were selected in the present study and they have an adequate structural variability and a large inhibitory activity range. First, the selected inhibitors were docked into G6PD in a bound state. Then molecular dynamics (MD) simulations and binding free energy calculations were performed to refine the binding structures and to understand the structure–activity relationship of the G6PD inhibitors. A detailed analysis of the determined binding modes and binding free energies provides valuable insights into the structure–activity relationship and may guide future design of more potent G6PD inhibitors.

2. Computational details

2.1. Structure preparation

The initial model of G6PD was constructed on the basis of the crystal structure of the complex of human G6PD with G6P (PDB code 2BHL) [23]. All small molecules in the crystal structure were removed except the crystal water molecules. The G6PD inhibitors examined in the present study are steroid DHEA derivatives [22]. Their molecular structures and IC_{50} values are listed in Table 1. The partial atomic charges for the atoms in these inhibitors were calculated by using the RESP protocol implemented in the Antechamber module in AMBER9 package [24,25] after electrostatic potential (ESP) calculation at HF/6-31G* level using Gaussion03 program [26].

2.2. Molecular docking

The docking program AUTODOCK4.2 with the genetic algorithm method was used to perform the automated molecular docking to explore the binding mode of G6PD with the molecules. All hydrogen atoms in the enzyme were removed except the polar hydrogen atoms. The grid box dimensions were set as 60 Å × 60 Å × 60 Å around the active site and the grid spacing was 0.375 Å. GA population size and maximum number of energy evaluations were set as 150 and 250,000, respectively. The docked structures were examined, and the best pose for each inhibitor was selected on the basis of the docking score, the scaffold conformation and hydrogen bonds formed between the active site residues and the inhibitor. Finally, the inhibitor conformation with the highest top-score was subjected to energy minimizations and MD simulations.

2.3. Molecular dynamics simulation

All missing hydrogen atoms and Na^+ counterions were added by LEaP module in AMBER9 package [24,25]. After that each system was solvated in an orthorhombic box of TIP3P water molecules [27] with a minimal solute–wall distance of 10 Å. The prepared system was fully energy minimized followed by the equilibration through gradual increase of the temperature from 10 to 298.15 K. The production MD simulation was subsequently kept running for ~10 ns. During MD simulation, the time step was 2 fs and the cutoff value for nonbond interactions was 10 Å. The Shake procedure [28,29] was employed to constrain all bonds involving hydrogen atoms. All MD simulations were performed by the Sander module in AMBER9 package [24,25].

2.4. Binding free energies calculations

The Molecular Mechanics–Poisson–Boltzmann Surface Area (MM–PBSA) method [30] was used to calculate the binding free energies [31–34]. In MM–PBSA method, the free energy of the enzyme–inhibitor binding, ΔG_{bind}, is the difference between the free energies of protein-substrate complex (G_{cpx}) and the unbound receptor/protein (G_{rec}) and ligand (G_{lig}) as following:

$$\Delta G_{bind} = G_{cpx} - G_{rec} - G_{lig}. \quad (1)$$

The binding free energy (ΔG_{bind}) is the sum of the changes in the molecular mechanical (MM) gas-phase binding energy (ΔE_{MM}), solvation free energy (ΔG_{solv}), and entropic contribution ($-T\Delta S$):

$$\Delta G_{bind} = \Delta E_{MM} + \Delta G_{solv} - T\Delta S \quad (2)$$

The molecular mechanical energy ΔE_{MM} is further divided into the internal energy (ΔE_{int}), the Coulomb energy (ΔE_{ele}), the van der Waals energy (ΔE_{vdW}) in gas phase:

$$\Delta E_{MM} = \Delta E_{int} + \Delta E_{ele} + \Delta E_{vdW} \quad (3)$$

The solvation free energy is divided into a polar part (ΔG_{PB}) and a nonpolar part (ΔG_{np})

$$\Delta G_{solv} = \Delta G_{PB} + \Delta G_{np} \quad (4)$$

Here, the polar contribution (ΔG_{PB}) is calculated by solving the Poisson–Boltzmann (PB) equation [35] as implemented in AMBER9 package [24,25]. The value of the interior dielectric constant and exterior dielectric constant were set to 1 and 80, respectively. The nonpolar solvation energy (ΔG_{np}) was calculated from the solvent-accessible surface area (SASA) using the hard-sphere atomic model. The probe radius of the solvent was set to 1.4 Å. ΔG_{np} is calculated using

$$\Delta G_{np} = \gamma \cdot \Delta SASA + \beta. \quad (5)$$