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Molecular docking and molecular dynamics simulation studies of GPR40 receptor–agonist interactions

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ABSTRACT

In order to explore the agonistic activity of small-molecule agonists to GPR40, AutoDock and GROMACS software were used for docking and molecular dynamics studies. A molecular docking of eight structurally diverse agonists (six carboxylic acids (CAs) agonist, and two non-carboxylic acids (non-CAs) agonist) was performed and the differences in their binding modes were investigated. Moreover, a good linear relationship based on the predicted binding affinities (pK_i) determined by using AutoDock and experimental activity values (pEC50) was obtained. Then, the 10 ns molecular dynamics (MD) simulations of three obtained ligand–receptor complexes embedded into the phospholipid bilayer were carried out. The position fluctuations of the ligands located inside the transmembrane domain were explored, and the stable binding modes of the three studied agonists were determined. Furthermore, the residue-based decomposition of interaction energies in three systems identified several critical residues for ligand binding.

1. Introduction

Type 2 diabetes, accounting for 90–95% of all diabetes, is linked to obesity and is characterized by improper insulin secretion and insulin resistance [1]. Diabetes leads to several complications including cardiovascular disease, diabetic retinopathy, lipid disorders, and hypertension. It is estimated that there would be, by 2010, as many as 220 million people in the world projected to suffer from this debilitating disease [2]. Since glycemia control of type 2 diabetes often deteriorates in spite of aggressive treatment, there is today an urgent active search for novel therapy. An important strategy for treatment of type 2 diabetes is to stimulate insulin secretion.

The G-protein coupled receptor GPR40, recently named free fatty acid receptor 1 (FFAR1), is predominantly expressed in human and rodent pancreatic islets and is activated by physiological concentrations of medium- and long-chain free fatty acids (FFAs) found in plasma such as linoleic and palmitic acids [3]. Recently, three independent researches provide evidences that FFAs amplify the glucose-stimulated insulin secretion (GSIS) by the pancreatic β-cell through the activation of GPR40 [3–5]. Given the need for novel treatments for type 2 diabetes, GPR40 represents a potentially attractive target. Acute administration of FFAs stimulates insulin release. Conversely, chronic exposure to high levels of FFAs leads to the impairment of β-cell function and lipotoxicity [6]. Hence, it is unclear whether the agonists or antagonists of GPR40 could be applied to the treatment of type 2 diabetes. The development of both potent agonists and antagonists of GPR40 are therefore required so that the detailed mechanism of the receptor in glucose balance may be unraveled. However, more recent researches are in favor of agonist therapy [7–9].

A large number of synthetic agonists of GPR40 have been proposed during the past 5 years [10–15]. Generally, the structure of the carboxylic acids (CAs) is used as a starting point for the design of new agonists. Additionally, some non-carboxylic acids (non-CAs) were proposed as effective agonists [16,17]. Recently, Bharate et al. have published reviews on progress in the discovery and development of small-molecule modulators of GPR40 [18]. They divided small-molecule GPR40 agonists into seven chemical classes.

Like other G-protein coupled receptors belonging to the same cluster of family A GPCRs, GPR40 are membrane proteins. All of them share a common structural motif of seven putative α-helical transmembrane spanning regions connected by three extracellular and three intracellular hydrophilic loops, an extracellular N terminus and intracellular C-terminal tail [19]. Such macromolecules are not easily amenable to crystallization and, therefore, to

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precise structure elucidation via X-ray diffraction. Thus, research focus has been on alternative techniques, such as, molecular modeling of GPCRs, either through homology modeling technique, or *de novo* model building, in order to derive reasonable 3D structures which can be used in structure-based drug design. Recently, Tikhonova et al. published the first structural model of the binding site of GPR40 complex with GW9508, which was obtained through a bidirectional, iterative approach that composed of molecular modeling and site-directed mutagenesis [20]. Twelve residues were identified within the GPR40 binding pocket.

Elucidation of ligand binding mechanisms is the necessary step to obtain more selective and potent drugs for this new potential target. Up to now, the correlation of agonist structure and its agonistic activity, the binding modes of non-CA agonists and GPR40, the binding energy, and the dynamics stability are still unknown. Therefore, in this paper, we made a molecular docking and molecular dynamics study to locate the binding site, get their dynamics information, and further identify the critical amino acid residues for ligand binding.

2. Methods and materials

2.1. Small-molecules preparation

We mainly selected eight small-molecule agonists which bear structure diversity including six classes of GPR40 agonists (class iii of GPR40 agonists is eliminated as they lack exact activity values). In order to get the most stable agonists conformations, the structure-optimizing calculation was carried out at the 6-31G** level by employing the Becke three-parameter Lee–Yang–Parr (B3LYP) hybrid density functional theory using the quantum chemistry software Gaussian 03 [21], and the structures with the lowest energy were selected for the following docking study. When docking, the Gasteiger–Hückel atomic charge was chosen for small-molecule agonists.

2.2. GPR40 model preparation

The homology model for GPR40 constructed based on the 2.65 Å resolution structure of rhodopsin (PDB code 1GZM) has been reported by Tikhonova et al. [20]. The binding site for GW9508 was experimentally verified and the coordinates of the validated GPR40 complex with GW9508 are available online [20]. Thus, we employ the Tikhonova's trusted model in the molecular docking study. The GW9508 was deleted and the GPR40 structure was then used in the docking experiments.

2.3. Molecular docking

Molecular docking of CA and non-CA agonists to the GPR40 model was carried out using the AutoDock3.0.5 software package [22]. All the torsion angles in the small-molecules were set free to perform flexible docking. Polar hydrogen was added by using the Hydrogen module in AutoDock Tools (ADT) for GPR40. After that, Kollman united atom partial charges were assigned for the receptor.

The empirical free energy function and Lamarckian genetic algorithm (LGA) were used for docking with the following settings: a maximum number of 25,000,000 energy evaluations, an initial population of 150 randomly placed individuals, a maximum number of 27,000 generations, a mutation rate of 0.02, a crossover rate of 0.80 and an elitism value (number of top individuals that automatically survive) of 1. For the local search, the so-called Solis and Wets algorithm was applied with a maximum of 300 iterations per search. Fifty independent docking runs were carried out for each ligand. Results were clustered according to the root-mean-square deviation (RMSD) criterion. The best docked conformations of small-molecule agonists were selected as initial active/binding conformations to evaluate potential correlations between experimental activities and predicted log K_i values.

2.4. Molecular dynamics simulations

Molecular dynamics (MD) simulations were performed using the GROMACS 3.3.3 package with the standard GROMOS96 force field [23,24]. The obtained complexes of GPR40 with three agonists (GW9508, compound **5** and compound **7**) were used for performing MD simulations. The model of the POPC (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylcholine) bilayer was used for simulation of the phospholipid environment around the receptor. The lipid parameters were taken from the literature [25–27]. The partial atomic charges of three agonists were calculated at DFT/B3LYP/6-31G** level using Gaussian 03 [21] package. Topology file and other force field parameters except the charges of ligands were generated using the PRODRG program [28]. Fig. 1, as an example, shows the GPR40-GW9508 complexes in the POPC/water systems. The three systems were neutralized by adding Cl^- counterions by replacing water molecules, respectively. The energy of these complexes was minimized using the steepest descent approach realized in the GROMACS package. Then, a 100 ps position restraining simulation was carried out to restrain the GPR40 by a 1000 kJ/mol Å^2 harmonic constraint to relieve close contacts before the actual simulation. Finally, three 10 ns MD simulations were performed at the NPT canonical ensemble and the periodic boundary conditions were used in all three dimensions. Phospholipids, water molecules, receptor, and ligand were coupled separately in a temperature bath at 300 K, with a coupling constant τ_t = 0.1 ps. The pressure coupling was set as independent in the *x* and *y* directions (semiisotropic coupling), with a constant pressure of 1 bar and a coupling constant τ_p of 1 ps [29]. The particle mesh Ewald (PME) method [30] for long-range electrostatics, a 14 Å cutoff for van der Walls interactions, a 12 Å cutoff for Coulomb interaction with updates every 10 steps, and the Lincs [31] algorithm for covalent bond constraints were used.

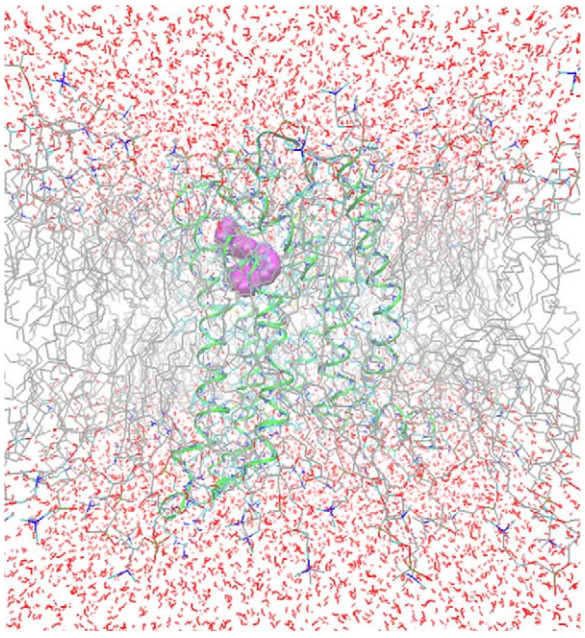

Fig. 1. Typical structure of GPR40 and GW9508 embedded in the hydrated lipid bilayer. The backbone of the receptor is represented in green, the POPC are in silver, water is in red and the agonist GW9508 is displayed as VDW in magenta.