Molecular Modeling of Quercetin Binding to the Peroxisome Proliferator-Activated Receptor-Gamma

Cristina COMAN (ISVORANU), Carmen SOCACIU

University of Agricultural Sciences and Veterinary Medicine, Faculty of Agriculture, Department of Chemistry and Biochemistry, Mănășturi St. 3-5, 400372 Cluj-Napoca, Romania

corresponding author: socaciucarmen@gmail.com

Abstract. The interaction between the quercetin (a generic flavonoid molecule) and the ligand binding domain of the peroxisome proliferator–activated receptor-gamma was investigated using the AutoDock software, which allows docking of molecular ligands to receptor macromolecules. AutoDock is able to find the most favourable binding site for quercetin on the receptor ligand binding domain and to predict the binding mode. The results show that the bonding is mainly driven through hydrogen bond type interactions and suggest the existence of two favourable quercetin conformations which coexist.

Keywords: AutoDock, docking, rutin, quercetin, PPAR-γ receptor.

INTRODUCTION

The peroxisome proliferator-activated receptors (PPARs) are a class of nuclear receptors. The interest in the PPARs originates from the fact that they are key regulators in adipocyte differentiation, and in lipid and glucose metabolism (Desvergne et al., 2004; Ehrmann et al., 2002).

As opposed to signalling molecules that function as ligands for transmembrane receptors (i.e. polypeptidic hormones) and exert their action by binding to the receptors and then inducing a cascade of intracellular biological responses, the ligands for nuclear receptors (i.e. steroid hormones) act directly on the nucleus and signal transduction takes place without intermediates (Socaciu, 1996). More precisely, the ligand binding to a nuclear receptor activates the receptor, which then has the ability to directly bind to DNA and regulate gene expression (up- or down-regulation of gene expression), reason for which they are called transcription factors.

Three PPARs subtypes have been identified in mammals: PPAR-α, PPAR-γ, and PPAR-δ (known as PPAR-β) (Ehrmann et al.). Common ligands for PPARs are saturated and unsaturated fatty acids and fatty acid metabolites (Ehrmann et al., 2002; Kasuga et al., 2007), as well as synthetic ligands. For example, the PPAR-γ is commonly used as target for the treatment of type 2 diabetes mellitus (Goldstein, 2008). This receptor is expressed in tissues where insulin exerts its action: adipocytes, myocytes, but also macrophages and vascular smooth muscles and plays a key role in the process of insulin sensitisation (Goldstein, 2008; Nunn et al., 2007). The antidiabetic drugs from the thiazolidinediones class (i.e. pioglitazone, rosiglitazone) are ligands that exert their effect by binding to the peroxisome proliferator-activated receptor-γ (PPAR-γ receptor) and activating the receptor. The activation of PPAR-γ results in a reduction of plasma non esterified free fatty acids (fat redistribution from liver and muscle cells towards adipose tissue cells), leading to improved muscle insulin sensitivity and
insulin signalling by increasing the insulin-stimulated GLUT4 receptor activity and muscle glycogen synthesis (Petersen et al., 2006).

In the present study quercetin will be investigated as potential ligand for the PPAR-γ receptor. The focus will be on modeling the interaction between the ligand quercetin (Fig. 1(a)) and the ligand binding domain (LBD) of the PPAR-γ receptor (Fig. 2) using the molecular docking AutoDock software. Identifying new small molecule ligands for PPAR-γ is a research area of interest which may have an impact on the management and treatment of type 2 diabetes mellitus.

The interest in quercetin stems from the fact that it is the aglycone part of the rutin molecule, which is mentioned in literature as a potential antidiabetic compound (http://www.ars-grin.gov/duke/; Ahmed et al., 2010; Kamalakkannan et al., 2006; Fernandez et al., 2010). Rutin (quercetin-3-O-rutinoside) is a flavonoid molecule, formed of quercetin and rutinoside (β-D-glucose and α-L-rhamnose) moieties (Fig. 1(b)), as the name suggests. According to literature (Pashikanti et al., 2010), rutin is not absorbed intact by the body, but it is metabolised in the intestine to quercetin and phenol derivatives, which implies that the potential antidiabetic effect is caused by the metabolites rather than rutin itself. The mechanism of action of rutin is not entirely understood. A recent experimental study (Ahmed et al., 2010) links its hypoglycaemic effect of rutin on nicotinamide-streptozotocin-induced diabetic rats with increased expression of the PPAR-γ receptor. For this reason, the present work aims at modeling the interaction between quercetin and the LBD of the PPAR-γ. The LBD is the active part of the any macromolecule, where any ligand is expected to bind.
Molecular docking is a very important tool, specially in computer-aided drug design. The AutoDock software (http://autodock.scripps.edu/) is a suite of programs that makes it possible to perform protein-ligand docking, more precisely it allows predicting how the two molecules interact, which is the prefered orientation of the ligand molecule with respect to the receptor, the type and strength of the bonding. It is particularly useful to predict how small molecule, potential drug candidates bind to receptors of known structure.

MATERIALS AND METHODS

The molecular docking calculations were conducted using the AutoDock software version 4.2 (Morris et al., 2009). The AutoDock 4.2 used for the present study consists of two programs: AutoGrid which pre-calculates some grid maps which are used for describing the target protein or the target binding site in the protein molecule, and AutoDock which performs the actual docking simulation of the ligand to these grids. In order to setup the molecules for docking, to run the actual docking calculations, and also to visualise the docking results, a graphical user interface called AutoDock Tools (ADT) is used (Sanner et al., 1999).

AutoDock performs calculations on molecules with known structures. Normally, the receptor structures are experimentally determined by x-ray crystallography and can be imported from international validated databases. The 3D molecular structure of the LBD of PPAR-γ was obtained from the Protein Data Bank [http://www.pdb.org] (pdb code 1PRG). The 1PRG molecule is a dimer build up of two chains A and B, each consisting of 270 amino acid sequences (residues 207-476 of the whole PPAR-γ receptor) (Nolte et al., 1998). For the calculations, the B chain of the dimer was deleted from the 1PRG structure, in order to have only one possible active binding site for the quercetin molecule.

Prior to docking, the correct input files needed to run the AutoGrid and AutoDock calculations are set up using the ADT graphical user interface. These preparations refer to: removing any water and solvent molecules present in the original Protein Data Bank files, merging non-polar hydrogens (only polar hydrogens are used by AutoDock), adding Gasteiger charges, choosing the active torsions in the ligand molecule, assigning atom types in the ligand molecule, setting up the grid parameter files and docking parameter files. A rigid receptor was used for the calculatins and for the quercetin ligand the number of free torsions was six (all possible six active torsions were allowed to move).

The affinity grid maps were generated by using the auxiliary program AutoGrid. Because the binding site for quercetin is not known, the calculations were performed in two consecutive steps. First, in an initial calculation the grid box size was set 126x126x126 points with 0.375 Å separation in between the grid points, which covers almost all the surface of the PPAR-γ LBD. In this initial experiment the macromolecule and the ligand are placed in random positions; when the docking job starts AutoDock places the ligand inside the volume of the grid box and finds the most favourable binding site in the receptor. Once the prefered binding site was identified, in a second step more refined calculations were carrried out, by changing the initial quercetin ligand coordinates with those of the lowest energy conformation found in the initial AutoDock run. This time a grid box of 60x60x60 points, centered on the ligand, with 0.375 Å distance in between the points was used.

The Autodock simulations were carried out using the Lamarckian Genetic Algoritm (Morris et al., 1998). Default parameters were used, except for the number of energy
evaluations and the number of trials. The number of GA runs (genetic algorithm runs) was set to 100, and different docking calculations were carried out with the maximum number of energy evaluations of 250,000, 2,500,000, and 10,000,000 respectively. The resulting docked quercetin conformations were clustered into groups of similar binding modes, with a root mean square deviation (rmsd) of 2.0 Å.

RESULTS AND DISCUSSION

The AutoDock job is composed of several runs or trials (GA runs). For each run there is a maximum number of energy evaluations that is performed. After each docking run, AutoDock gives ligand lowest energy conformation it finds after performing the run. The cartesian coordinates and different energy values are given as output. At the end of the job, several conformations are calculated, depending on the number of docking runs. The conformations are grouped in clusters, according to their similarity to each other. The similarity is measured in terms of root mean square deviation between the coordinates of the different atoms in the ligand. The clustering results obtained for the different autodock jobs performed in this study, at an rms value of 2.0 Å, are shown in Fig. 3. The clustering improves with the number of energy evaluations (see also Fig. 4); the best clustering was obtained for the most refined job performed, namely 100 GA runs and 10,000,000 energy evaluations (Fig 3(c)).

![Fig. 3.](image)

There are two clusters (labelled A and B) grouping quercetin conformations of around -7.5 kcal/mole and -7.2 kcal/mole binding energies, which for the best docking of 10,000,000 evaluations have roughly the same population (no. of conformations from each cluster) of 41% A and 42% B. Normally, the best docking result is the configuration with lowest energy, which is the most stable configuration and which is expected to have the highest population. In the particular case here considered of rutin-PPAR-γ LBD docking, it is concluded that...
since the A and B clusters have very similar populations, actually two conformations giving rise to the clusters are both favourable conformations. Since they are close in binding energy, it is likely that both conformations are energetically allowed. Representative docking parameters for the two clusters are shown in Tab. 1.

Representative docking results obtained for the quercetin-PPAR-γ LBD system. The binding energies and docking energies (\(E_{\text{binding}}\) and \(E_{\text{docking}}\)) are given for the lowest energy configuration in each cluster. The binding energy is defined as the sum of intermolecular and torsional energies, and the docking energy is given by the sum of intermolecular and internal energy for each system. The CPU time is the total time required for performing the autodock job.

<table>
<thead>
<tr>
<th>Cluster</th>
<th>E evaluations</th>
<th>GA runs</th>
<th>(E_{\text{binding min}}) (kcal/mole)</th>
<th>(E_{\text{docked min}}) (kcal/mole)</th>
<th>% of total population</th>
<th>CPU time</th>
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<tbody>
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<td>A</td>
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<td>-7.49</td>
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<td>16h20m25s</td>
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</table>

In the following paragraphs, the lowest energy conformations from the A and B clusters will be named configuration A and configuration B and the discussion will focus on the results obtained for the 100 GA runs and 10.000.000 energy evaluations docking since it is the one that gives best results. In Fig. 5 the receptor-ligand complex for both quercetin configurations is illustrated, as well as their relative orientation to eachother. As can be seen from the figure, the quercetin binding site is located in the proximity of the \(\beta\)-strands of the PPAR-γ LBD.

![Fig. 5. The quercetin-PPAR-γ LBD complex for (a) conformation A and (b) conformation B, as resulted from the AutoDock calculations; (c) the relative orientations of conformations A and B rotated with respect to what is shown in panels (a) and (b), for a better view of the structures.](image)
The quercetin-PPAR-γ LBD interaction is mostly driven by hydrogen bonds: the intermolecular energy term that accounts for van der Waals and hydrogen bond type interactions weights more than 90% of the total intermolecular interaction (94.3% for conformation A and 91% for conformation B), as opposed to the electrostatic term which is below 10%. Five hydrogen bonds are formed in between hydroxyl groups of quercetin in conformation A and amino acid residues in the receptor, while conformation B interacts by four hydrogen bonds (Tab. 2 and Fig. 6).

The hydrogen bonds formed in between the quercetin molecule and the LBD of the PPAR-γ receptor, as resulted from the AutoDock calculations. The H bond donor is considered the molecule which donates the proton, while the H bond acceptor is the molecule containing the electronegative element.

<table>
<thead>
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<th>H bond donor</th>
<th>H bond acceptor</th>
<th>H bond donor</th>
<th>H bond acceptor</th>
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<tr>
<td>QUE (H)</td>
<td>GLU291 (O1)</td>
<td>SER342 (NH)</td>
<td>QUE (O)</td>
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<td>GLU291 (O2)</td>
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</tr>
</tbody>
</table>

Tab. 2

Fig. 6. Illustration of the hydrogen bonds formed between the quercetin molecule and the LBD of the PPARγ receptor (a) binding mode for configuration A and (b) binding mode for configuration B.

The amino acid residues of the receptor involved in the interaction with conformation A are the SER342, LEU228, MET329, and GLU291, while conformation B interact with SER342, LEU228, GLU291, and GLU343. More precisely, with conformation A the GLU291 residue forms two H bonds in which it acts as H bond acceptor, MET329 one bond as acceptor, SER342 and LEU228 form each one bond as donors. On the other hand GLU291 forms only one H bond with conformation B, and SER342, LEU228 and GLU343 form the other three bonds as donors with the B conformation.
CONCLUSIONS

By using the AutoDock software it was possible to investigate the interaction between quercetin as ligand and the LBD of the PPAR-γ receptor. The influence of improving the docking parameters (the number of energy evaluations per docking run) on the quality of final results, especially on the clustering was also tested. Two favourable quercetin conformations of close energies, named conformations A and B were reported in the present study. Quercetin binds to PPAR-γ by hydrogen bond type interactions; conformation A forms five hydrogen bonds with the receptor, while conformation B forms four hydrogen bonds. Since the exact mechanism of action of the hypoglycaemic flavonoid rutin is not yet understood and since it is believed that it exerts the action through metabolites such as quercetin, the present study is of particular interest. In the future more work will be carried out by performing more extensive calculations in order to try to find out if any of the two most stable conformations becomes predominant.

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