Docking Studies of Chlorogenic Acid against Aldose Reductase by using Molgro Virtual Docker Software

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INTRODUCTION

Chlorogenic acid (5-O-caffeoylquinic acid), a phenolic compound found ubiquitously in plants, is an antioxidant and metal chelator (Kono et al., 1998). It has been reported that the intake of this compound in diabetic rats, lowered glucose concentration (Andrade-Cetto and Wiedenfeld, 2001; Rodriguez de Sotillo and Hadley, 2002) and the intake of Cecropia obtusifolia leaf extracts, that are rich in chlorogenic acid, resulted in a significant reduction in plasma glucose concentrations in persons with type 2 diabetes (Herrera-Arellano et al., 2004). Although, various preventive effects of chlorogenic acid on diabetes were reported but the effects of this compound on diabetic complications are still unknown. Therefore, the effect of chlorogenic acid on aldose reductase, an enzyme involved in diabetic complications such as nephropathy (Sato et al., 1992, Dunlop et al., 2000), neuropathy (Oates et al., 1997), cataract formation (Lee et al. 1995) or angiopathy (Jay et al., 2006) has been studied here.

Aldose reductase (EC 1.1.1.21, AR), first and the rate limiting enzyme of the polyol pathway converts glucose to sorbitol, in the presence of NADPH as reducing cofactor. The second enzyme of the pathway, sorbitol dehydrogenase (EC 1.1.1.14), oxidizes sorbitol to fructose with NAD as cofactor (Yabe-Nishimura et al., 1998; Brownlee et al., 2001). Various studies have showed that the patho-physiological activity of aldose reductase (AR) plays a key role in the development of diabetic complications (Yabe-Nishimura et al., 1998; Kador et al., 1988; Tomlinson et al., 1994; King et al., 1996). So, a possible strategy to prevent the onset and progression of the diabetic complications is to inhibit this enzyme (Miyamoto et al., 2002; Costantino et al., 2000; Srivastava et al., 2005; Costantino et al., 1999). Several crystal structures of aldose reductase have been solved by X-ray diffraction complexed with NADPH (Figure 1) and diverse aldose reductase inhibitors (ARIs). All these crystal structures have shown that the active site of this enzyme is a large, deep, ellipsoidal cavity, approximately 4 Å x 15Å wide and 15 Å deep, wherein the nicotinamide ring of the NADPH cofactor and the substrate-binding site are found. The binding site is divided into 2 sub-pockets (Figure 2): an anion binding site and a specificity pocket.
The anion binding pocket is formed by hydrophilic residues (Tyr48, His110, Trp111) and the 4-pro-R-hydrogen of the nicotinamide ring of NADPH and involved in catalysis, whereas, the second, specificity pocket is formed by hydrophobic residues Trp111, Trp20, Trp79, Ala299, Leu300 and Phe122. (Yabe-Nishimura et al., 1998; El-Kabbani et al., 1997, Nakano et al., 1996). It can adopt various conformations depending on the size and properties of the bound ligand and is responsible for the various substrate and inhibitor specificities. (Harrison et al., 1997). Hydrogen bonds are formed between the polar groups of the inhibitor and the active-site residues Tyr48, His110, and Trp111 AR (Wilson et al., 1993; Urzhumtsev et al., 1997; Harrison et al., 1997; Rogniaux et al., 1999; Oka et al., 2000).

Thus, the inhibitor binding site is a positively charged anion well formed by Tyr48, His110 and the nicotinamide ring (Harrison et al., 1997; Harrison et al., 1994) and the required structural elements of aldose reductase inhibitors are proposed as an aromatic ring system to form hydrophobic or π-π stacking interactions with the hydrophobic amino acid residues in the active site (Tyr20) and an acidic ionisable group such as those of carboxylic acids and spirohydantoin which can anchor to the anionic binding site (Asp43/Lys77'/?Tyr48'/?NADP' complex (Miyamoto et al., 2002a; Lee et al., 1998; Sun et al., 2003).

During this study, chlorogenic acid have been docked into the active site of aldose resuctasate by using the Molgro Virtual Docker (MVD) software. Docking methods typically use an energy-based scoring function to identify the most energetically favorable conformation of a ligand when bound to the macromolecular target. Lower energy scores indicates more favored protein-ligand complexes. Molecular docking is thus an optimization problem, where the task is to find the ligand binding mode with lowest potential energy. The process of docking involves sampling the coordinate space of the target binding site and scoring each possible ligand pose within that site, the highest scoring pose then taken as the predicted binding mode for that compound. There are many different docking programs now available and they differ in the nature of the sampling algorithms they employ, in their manner of handling ligand and protein flexibility, in the scoring functions they use, and in the cpu time they required. Some of the most widely used docking programs include: Gold (Jones et al., 1995, 1997), Dock (Ewing et al., 2001), FlexX (Rarey, et al., 1996; Kramer et al., 1999), Glide (Halgren et al., 2004; Friesner et al., 2004), Fred (McGann et al., 2003), LigandFit (Venkatachalam et al., 2003), Slide (Schnecke et al., 2000), AutoDock (Morris et al., 1998), ICM (Abagyan et al., 1994), QXP (McMartin et al., 1997), Surfle (Jain, 2003) and MVD (Thomsen et al., 2006).

Each of these programs attempts to predict the most likely structures of protein-ligand complexes and then to quantify the free energy of ligand binding, at the same time keeping the computing time within acceptable limits to allow for a rapid docking of large chemical libraries. In the studies reported here, MVD was used, because it showed higher docking accuracy when benchmarked against other available docking programs (MD: 87%, Glide:82%, Surfle:75%, FlexX:58%) (Thomsen et al., 2006) and has been shown to be successful in several recent studies (Pripp, 2007; Sapre et al., 2008; Chauhan et al., 2009; Paul et al., 2010), but also for reasons of cost and user-friendliness. In most docking studies, the target proteins are assumed to be rigid objects. This major assumption (neglecting protein flexibility) can cause errors (Carlson et al., 2000) and lead to inaccurate binding pose (Cavasotto et al., 2004; Osterberg et al., 2002; Murray et al., 1999). Proteins are flexible and dynamic objects and using a single protein conformation ignores important dynamic aspects of protein ligand binding (Jorgensen et al., 1991). Dealing with protein flexibility is one of the major challenges in the development of docking methods (Klebe et al., 2006; Sousa et al., 2006; Teague et al., 2003) - because of the high...
dimensionality of the search space involved and because of the complexity of energy function to be computed. Thus, a number of attempts have been made to introduce protein flexibility into docking protocols (Carlson et al., 2002; Klebe et al., 2006; Guvench et al., 2009), among which the use of multiple protein crystal structures is considered the best option to take advantage of the full flexibility of the receptor (Carlson et al., 2000).

MATERIAL AND METHODS

In this study, docking of chlorogenic acid against AR have been performed using MVD software. Usually, a single rigid crystal structure of an enzyme is used for docking studies, but here in order to incorporate the protein flexibility in this docking studies, five different crystal structures of AR, were selected (Table 1), from the available 95 crystal structures of AR (June 2009) held in the Protein Databank (PDB) (Deshpande 2005) accessed at the URL: (http://www.rcsb.org/pdb), - under the criteria that they had a reasonable resolution (≤ 2.8 Å) and involved the non-mutated aldose reductase enzyme from Homo sapiens, in complex with different ligands.

Chlorogenic acid have been docked against each of these five selected AR crystal structures and 10 independent runs were performed with the guided differential evolution algorithm, with each of these docking runs returning one solution (pose). The MolDock scoring function used by MVD is derived from the PLP scoring functions originally proposed by Gehlhaar et al. (1995, 1998) and extended later by Yang et al. (2004).

The 10 solutions obtained from the 10 independent docking runs were re-ranked, in order to further increase the docking accuracy, by using a more complex scoring function. In MVD, along with the docking scoring function terms, a Lennard Jones 12-6 potential (Morris, 1998) and sp²-sp² torsion terms were also used. On the basis of pilot docking studies, the MolDock re-rank scores were selected for ranking the inhibitor poses, and for all the aldose reductase docking performed here, the poses selected as the best were taken as those with the highest MolDock re-rank score. Aldose reductase crystal structures were directly downloaded to the workspace of MVD from the PDB (Deshpande, 2005) accessed at the URL: (http://www.rcsb.org/pdb). The structure of chlorogenic acid has been drawn on ChemDraw software and imported to the MVD workspace in ‘sdf’ format. In order to make accurate predictions, it is important that the imported structures have been properly prepared, that is, the atom connectivity and bond orders are correct and partial atomic charges are assigned. PDB files often have poor or missing assignment of explicit hydrogens, and the PDB file format cannot accommodate bond order information. All necessary valency checks and H atom addition were thus performed using the utilities provided in MVD. The binding site specifies the region of interest where the docking procedure will look for promising poses (ligand conformations). MolDock automatically identifies potential binding sites (also referred as cavities or active sites) by using its cavity detection algorithm. The cavities within a 30 x 30 x 30 Å cube centered at the experimentally known ligand position were used. The cavities that are identified by the cavity detection algorithm are then used by the guided differential evolution search algorithm to focus the search, to that specific area during the docking simulation. In the case of the crystal structures for aldose reductase complexes, the program generally identified five different binding sites (Figure 3). From these five predicted cavities the one with the highest volume (205.13 Å³) was selected for consideration, as it includes the bound ligand.

RESULTS AND DISCUSSION

The most convenient way to incorporate protein flexibility in the docking process is to perform docking using an ensemble of static receptor conformations and nowadays, this approach is applied more and more in virtual screening experiments (Bowman et al., 2007b; Cheng et al., 2008).

It is this approach that was applied here, using a collection of selected AR crystal structures with different bound ligands to provide different conformations of the protein and allow for structural changes in the protein upon ligand binding (Subramanian et al., 2006). Thus, five of the aldose reductase crystal structures bound to different ligands—2FZD, IUS0, 3G5E, 2INE and 2IKG were used in this study (Table 1).
Chlorogenic acid (Figure 4a) is formed by the esterification of caffeic acid and quinic acids. While docking of chlorogenic acid with the five crystal structures of aldose reductase, it is oriented in the active site such that it forms hydrogen bond with at least two of the three key residues i.e. Tyr48, His110 and Trp111.

Along with these three residues hydrogen bonds are also formed with some other active site amino acids among which Cys298 and Thr113 are most frequent.

The comparison of the binding of Tolrestat in the active site of aldose reductase (PDB ID: 2FZD) (Figure 5a) and docking of chlorogenic acid in the active site of the same enzyme (Figure 5b) clearly shows similarity in the binding of the two compounds.

Table 1: List of crystal structures of aldose reductase used for docking studies (PDB ID = four character PDB identifier representing each entry).

<table>
<thead>
<tr>
<th>PDB ID</th>
<th>Ligand</th>
<th>Ligand structure</th>
<th>Ligand volume (Å)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1US0</td>
<td>IDD594</td>
<td></td>
<td>921.8</td>
<td>Howard, 2004</td>
</tr>
<tr>
<td>2FZD</td>
<td>Tolrestat</td>
<td></td>
<td>860.2</td>
<td>Steuber, 2006</td>
</tr>
<tr>
<td>2IKG</td>
<td>Nitrophenyl oxadiazole type inhibitor</td>
<td></td>
<td>729</td>
<td>Steuber, 2007</td>
</tr>
<tr>
<td>2INE</td>
<td>Phenylacetic acid</td>
<td></td>
<td>480.8</td>
<td>Brownlee, 2006</td>
</tr>
<tr>
<td>3G5E</td>
<td>IDD740</td>
<td></td>
<td>898.2</td>
<td>Van Zandt, 2009</td>
</tr>
</tbody>
</table>

Table 2: MVD and Re-rank score (kcal/mol) for chlorogenic acid when docked with five aldose reductase crystal structures.

<table>
<thead>
<tr>
<th></th>
<th>MolDock Score</th>
<th>MolDock Re-rank Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>2FZD</td>
<td>-107.75</td>
<td>-101.48</td>
</tr>
<tr>
<td>2IKG</td>
<td>-92.35</td>
<td>-91.368</td>
</tr>
<tr>
<td>2INE</td>
<td>-122.83</td>
<td>-119.73</td>
</tr>
<tr>
<td>3G5E</td>
<td>-132.78</td>
<td>-127.71</td>
</tr>
<tr>
<td>1US0</td>
<td>-140.99</td>
<td>-134.32</td>
</tr>
<tr>
<td>Average</td>
<td>-119.34 ± 19.5</td>
<td>-114.92 ± 18</td>
</tr>
</tbody>
</table>

(Kinoshita et al., 2002; Steuber et al., 2007; Steuber et al., 2006; Van Zandt et al., 2009; Howard et al., 2004.

Chlorogenic acid was docked with all of these five selected human crystal structures of AR bound to different ligands. In each docking run, the best poses were selected on the basis of their MVD re-rank scores and the mean of the 5 re-rank scores was then computed as the final score for each compound.

The MVD score and the re-rank scores of the best poses for each of the docking studies of chlorogenic acid with five crystal structures of AR, and their average is presented in Table 2. The average MolDock score and the MolDock Re-rank score obtained for chlorogenic acid are -119.34 Kcal/mol and -114.92 Kcal/mol respectively. The best docking poses obtained on the basis of MVD re-rank score for chlorogenic acid with each of the 5 crystal structures of aldose reductase are presented in Figures 4.

The reported crystallographic studies have shown that the interactions of ligands in the catalytic site of AR are mostly polar (Howard et al., 2004), and in most of the AR crystal structures involving the more potent inhibition, these compounds are all carboxylic acids, with their carboxylate groups firmly anchored in the active site, with hydrogen bonds to His110, Tyr48, and Trp111.
Fig. 4: The best scored docking solution of Chlorogenic acid (a) with the five selected crystal structures of AR (b to f). (The coenzyme NADPH is removed for the sake of clarity. Amino acids in the active site are presented in ball and stick with element colour and ligand is presented in thick lines with element colour (where carbon is grey, oxygen is red, nitrogen is blue and sulphur is yellow and hydrogen in white). Green lines represent the hydrogen bonds in between the ligand and the active site of AR).
CONCLUSION

The docking studies detailed above provide estimates of the inhibitory activities of the docked ligand. The results show that chlorogenic acid fits well in the active site of aldose reductase and also interact with the residues in the active site which are important for their biological activity, thus, chlorogenic acid could be a putative inhibitor of aldose reductase and can be used to prevent the onset/treatment of diabetic complications.

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