Cross-docking study of flavonoids against tyrosinase enzymes using PyRx 0.8 virtual screening tool

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Introduction

Protein-ligand docking is an essential tool in studying and understanding of protein-ligand interaction.¹ Docking is used to predict protein-ligand binding mode and affinity.² There are many free docking programs that have been used in molecular modeling such as AutoDock, AutoDock Vina, PyRx and ArgusLab. To start the docking study, normally we begin with validation the docking protocol by re-docking of ligand found in protein from PDB file. It is a rapid way to evaluate a docking procedure before working with target ligands.³ However, this re-docking sometime can give misleadingly good results but obtaining poor target docking results. In multiple available PDB protein structures for receptor, selection of the best structure for pose prediction is an important step. So cross-docking is an alternative way to find the best holo structures among multiple structures available for a target protein.⁴ In this study, we performed cross-docking of three tyrosinase enzymes from RSCB and used AutoDock Vina in PyRx virtual screening tool⁵,⁶ as docking engine. PyRx is a virtual screening software for computational drug discovery that can be used to screen libraries of compounds against potential drug targets. PyRx enables medicinal chemists to run virtual screening from any platform and helps users in every step of this process which starts from data preparation to job submission and analysis of the results. PyRx includes docking wizard with easy-to-use user interface which makes it a valuable tool for Computer-Aided Drug Design (CADD). PyRx also includes chemical spreadsheet-like functionality and powerful visualization engine that are essential for rational drug design.

Materials and methods

Software and PDB files

To examine the cross-docking of binding orientation of substrates with tyrosinase protein, Autodock Vina in PyRx virtual screening tool PyRx 0.8 (http://pyrx.sourceforge.net/) was chosen for this study. For tyrosinase protein, crystal structures of tyrosinase (PDB ID: 5I3B, 4P6R, 4P6S) were retrieved from RCSB Protein Data Bank. Target structures and anti-tyrosinase activity data were obtained from review literatures by Chang S⁷. L-Dopa, tyrosine and hydroquinone were used as positive control compounds.

Preparation of the ligand structures

The ligands 2D structures quercetin, artocarpetin, streppogenin, dihydromorin, taxifolin, calycosin and haginin A were drawn in ChemSketch program (http://www.acdlabs.com/resources/freeware/chemsketch/). Then, the ligands 2D structures were converted to 3D structures and the structures were energy minimized using Avogadro package (http://avogadro.cc/wiki/Main_Page).
Cross-docking studies

Ligand-bound protein cross-docking

The apo protein of tyrosinase crystal structure (PDB ID: 5I3B, 4P6R, 4P6S) was opened in PyRx virtual screening tool as a starting protein structure in pdbqt format. The ligand was chosen, and automatically converted to pdbqt format. After both of them were chosen, the grid box was automatically appearing and the centre of the target site was assign along with the dimensions. The centres of the box were assigned for 5I3B (X = 2.76, Y = 99.84, Z = 23.17), 4P6R (X = 28.61, Y = 17.47, Z = 14.55), and 4P6S (X = -19.86, Y = 7.44, Z = -9.22) together with the exhaustiveness equalling to 8. The dimensions of the box were set to 25 × 25 × 25 Å. The docking was performed with autodock vina and re-docking ligand to observe the precision of the docking condition. In this study, one ligand was compared with three tyrosinase receptors. The best free energy of binding values would be obtained in PyRx virtual screening tool GUI and log files. The poses were chosen by compared the orientation with reference ligand x-ray structure of the certain enzyme. The RMSD was calculated using Vega-ZZ program (nova.disfarm.unimi.it). The DS Visualizer 4.0 was used to perform for all figures.

Flavonoids cross-docking

Seven anti-tyrosinase flavonoids were cross-docking with three tyrosinase enzyme in the same condition as previous study. The binding energy of the best pose from each compound was plot with the anti-tyrosinase activity (IC50) and constructed the regression line in order to compare among the other. The best tyrosinase enzyme will be selected for further studies.

Results

Comparison of accuracy and reproducibility of x-ray ligand cross-docking results

The accuracy of a docking result is normally evaluated by the RMSD between the experimentally docking-observed and the x-ray ligand, which usually used RMSD cut-off value in a range of 2 – 3 Å. The RMSD values of cross-docking result of 3 ligands in three different tyrosinase enzymes were shown in Table 1. The orientations of re-docking ligand into its enzyme were depicted in Figure 1 and 2.

Figure 1. (A) Tyrosine re-docking conformer overlays with x-ray ligand of 4P6R and (B) L-Dopa re-docking conformer overlays with x-ray ligand of 4P6S

Figure 2. Hydroquinone re-docking conformer overlays with x-ray ligand of 5I3B
Table 1 RMSD of re-docking and cross-docking

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Enzyme 4P6R</th>
<th>Enzyme 4P6S</th>
<th>Enzyme 5I3B</th>
</tr>
</thead>
<tbody>
<tr>
<td>4P6R_Tyrosine</td>
<td>0.255</td>
<td>0.358</td>
<td>0.091</td>
</tr>
<tr>
<td>4P6S_L-Dopa</td>
<td>0.160</td>
<td>0.133</td>
<td>0.068</td>
</tr>
<tr>
<td>5I3B_Hydroquinone</td>
<td>0.109</td>
<td>0.067</td>
<td>0.060</td>
</tr>
</tbody>
</table>

**Correlation between docking score and anti-tyrosinase activity**

In this study, we used data from Chang7 which collected IC50 of anti-tyrosinase activity of compounds from different sources and assigned by divided with IC50 of reference standard in those experiments which is kojic acid. After cross-docking flavonoids with 3 tyrosinase enzymes, the data and graphical analysis of docking scores versus anti-tyrosinase activities (IC50) were demonstrated in Table 2 and Figure 3 - 4.

Table 2. Ligand-enzyme binding energy of flavonoids and tyrosinase enzymes

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50 (F)*</th>
<th>Enzyme 4P6R Binding energy (kcal/mol)</th>
<th>Enzyme 4P6S Binding energy (kcal/mol)</th>
<th>Enzyme 5I3B Binding energy (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artocarpin</td>
<td>0.1</td>
<td>-7.6</td>
<td>-8.6</td>
<td>-8.4</td>
</tr>
<tr>
<td>Quercetin</td>
<td>0.2</td>
<td>-7.9</td>
<td>-8.2</td>
<td>-8.4</td>
</tr>
<tr>
<td>Dihydromorin</td>
<td>0.5</td>
<td>-6.8</td>
<td>-7.9</td>
<td>-7.9</td>
</tr>
<tr>
<td>Taxifolin</td>
<td>1.0</td>
<td>-7.1</td>
<td>-8.0</td>
<td>-8.0</td>
</tr>
<tr>
<td>Calycosin</td>
<td>1.3</td>
<td>-7.7</td>
<td>-7.5</td>
<td>-7.7</td>
</tr>
<tr>
<td>Haginin A</td>
<td>10.1</td>
<td>-6.9</td>
<td>-7.2</td>
<td>-7.0</td>
</tr>
<tr>
<td>Streppogenin</td>
<td>13.6</td>
<td>-5.4</td>
<td>-7.3</td>
<td>-7.3</td>
</tr>
</tbody>
</table>

*Compare to kojic acid (1F)

Figure 3. Correlation of docking score and anti-tyrosinase activities (IC50) of flavonoids and 4P6S (A), 4P6R (B)

Figure 4. Correlation of docking score and anti-tyrosinase activities (IC50) of flavonoids and 5I3B
Discussion

Accuracy and reproducibility of x-ray ligand cross-docking results

The accuracy of a docking result is normally evaluated by the RMSD between the experimentally docking-observed and the x-ray ligand, which usually used RMSD cut-off value in a range of 2 – 3 Å. The RMSD values of re-docking of tyrosine, L-Dopa and hydroquinone into their enzyme 4P6R, 4P6S and 5I3B were 0.255, 0.133 and 0.060, respectively. Meanwhile the RMSD values of cross-docking of tyrosine, L-Dopa and hydroquinone into other enzymes ranged from 0.060 to 0.160. Enzyme 5I3B gave the lowest RMSD values among 3 reference ligands.

Correlation between docking score and anti-tyrosinase activity

Using a set of flavonoids with known bioactivity, we evaluated the ability of cross-docking of these flavonoids into 3 tyrosinase enzymes to predict inhibition affinities. The value of the scoring function for a given ligand directly relates to its affinity for the target which often shows in a linear fashion. From the docking results, the best correlation was obtained from 5I3B enzyme with the highest correlation coefficient ($R^2 = 0.72$) while 4P6S and 4P6R gave the correlation coefficient ($R^2$) equal to 0.60 and 0.67, respectively.

Conclusion

Cross-docking experiment can differentiate the appropriate tyrosinase enzyme for docking process. Enzyme 5I3B gave the lowest RMSD values for 3 reference ligands and gave the best correlation with the highest correlation coefficient ($R^2$) equal to 0.72. In conclusion, 5I3B should be used as tyrosinase enzyme for docking experiment. The cross-docking protocol should be used for performing a good docking research in drug discovery.

References