CHAPTER 5

In silico analysis of interaction between apoptotic proteins and Isothiocyanates isolated from Brassica oleracea using docking studies

7.1 Introduction

Molecular docking is a structure-based computational technique that samples complementary fits of a macromolecular receptor and possible ligands. As the structures of more and more target receptors are determined, docking is increasingly used for lead discovery, typically by screening a large database of organic molecules for putative ligands that fit a binding site [350]. Docking program is designed to fit molecules in the site often in hundreds or even thousands of conformations, evaluates their complementarity and ranks each molecule relative to the rest of the database. Whereas the technique had some important successes in recent years, serious challenges remain [351]. These challenges may be divided into two categories: problems with calculating binding energies or complementarily scores and problems with sampling the degrees of freedom available to interacting molecules [352]. The isothiocyanates in crucifers has attracted much recent interest and found to possess anticancer, antioxidant and anti inflammatory properties. The mechanism by which these agents exert their anticancer action was reported to be apoptosis. Two major pathways leading to apoptosis exist in cells: the extrinsic pathway, which involves the activation of the death receptor family and the intrinsic pathway, which involves the mitochondria. In both the pathways, an apoptotic death stimulus results in the activation of caspases, the major executioners of this process, either directly or via activation of the mitochondrial death program. The p53 is a tumor suppressor that is essential for the prevention of cancer development and loss of p53 function is one of the early events in immortalization of human cells. There have been several successful applications of molecular docking studies in rational drug design, better the binding of receptor the better biological action will be elicited. In this study the binding of isothiocyanates with various apoptotic proteins such as p53, bax, bcl-2 and caspase 3 may induce the cancer cell to undergo apoptosis a natural recovery mechanism
exist in a cell. In our research we isolated sulforaphane from *Brassica oleraceae* studied its apoptosis induction through p53 which is mediated through bax and bcl-2 proteins.

7.2 Materials and Methods

7.2.1 Databases used in this study

Pubchem (pubchem.ncbi.nlm.nih.gov), Smiles online translator (http://cactus.nci.nih.gov/translate) used in this study are freely available for academic use. Molecular docking server was used on paid subscription. AutoDock (http://autodock.scripps.edu) is used for molecular docking calculations. Rasmol are used for visualisation of docking results. MMFF94 semi empirical method can be used to calculate small molecule geometries and electric properties. Detailed methodology can be accessed from server using an URL http://www.dockingserver.com/web/getting started. PDB files of various human proteins such as p53, bax, bcl-2 and casp-3 were obtained from Protein data Bank http://www.pdb.org.

7.2.2 Protein files preparation

Briefly, PDB files for p53 protein (IHS 5), bax (1F16) bcl-2(1MK3) casp-3 (1NM3) were downloaded from Protein Data Bank (http://www.pdb.org). All the proteins were uploaded to server. At protein clean step charge calculation method was selected as Gasteiger. All the protein chains were selected and hetero atoms are not removed as the binding site is not predicted. All water molecules were selected for cleaning. By completion of this step, protein clean, calculation of protein charges and solvation parameters as well as protein parameter file created [353]. In the next step a Grid (a three-dimensional box) was created with a dimension of X=20 Angstrom, Y=20 Angstrom, Z=20 Angstrom, while centre of mass was kept at a co-ordinate of X= 103.61, Y=100.67, Z=78.536. The protein was made ready for docking experiments.
7.2.3 Ligand files preparation

The Indole 3 carbinol and sulforaphane (SFN) structures were downloaded from Pubchem as .sdf files. The .sdf files were converted to .pdb files using smiles online translator. In the same way .sdf files of 5-fluorouracil were retrieved from pubchem and converted to .pdb files using smiles online translator. Molecular docking server was used for the preparation of ligand before docking experiment. Briefly, ligands were uploaded singly to server. Charge calculation and geometric optimization methods were selected as Gasteiger and MMFF94 respectively; while pH was kept as 7.0 [354]. By the end of this process ligand files are ready for the docking.

7.2.4 Docking of various apoptotic proteins with Isothiocyanates using AutoDock

Docking was started by selecting two ligands SFN, I3C and various proteins such as p53, bax, bcl-2 and casp-3 from their respective folders. The number of individuals in the population (ga_pop_size) was kept 150, AutoDock counts for numbers of energy evaluations (ga_num_evals) were kept 25000000 and the number of generations (ga_num_generation) selected as 540000. And rest other settings kept as default setting. Finally simulation experiment started with keeping the numbers of run as 100. AutoDock is the most popular molecular docking program is used for various molecular docking calculations. Many research reports supported the fidelity and accuracy of AutoDock docking tool [355-357]. Hence we considered Molecular Docking Server as an appropriate docking tool for this docking analysis of multiple proteins against our ligand isothiocyanates. AstexViewer and JMOL viewer (default viewer of docking server) and Rasmol viewer were used for the visualization of docking results.
7.3 Results

7.3.1 Docking of various apoptotic proteins with Isothiocyanates using Autodock

The binding energy for Isothiocyanates such as I3C and SFN with various human proteins such as p53, bax, bcl-2 and caspase 3 are tabulated in the Table 1. Frequencies of occurrence out of total population for p53 with SFN and 5FU were found to be the same as 18 % and for SFN and 5FU with bax, bcl-2 and caspase 3 were tabulated in the table. A comparison of different energies interacting surfaces and frequencies of species is shown in the table. Sulforaphane (1-Isothiocyanato-4-(methylsulfinyl)-butane) is converted to 4 isothiocyanate N – methyl butane1 –SO - thioperoxol by protonation in the ligand preparation process by docking server. In the examination of SFN binding with proteins SFN contains a hydrophilic sulfinyl group which forms a hydrogen bond with the aminoacids of protein. In general ITCs (isothiocyanate) including sulforaphane are electrophiles capable of modifying proteins thus initiating a cascade of events like apoptosis [20]. During docking a series of poses (ligand-protein complexes of particular conformation and mutual orientation) were generated for each molecule. The algorithm for the optimization of the ligand-protein orientation works by the alignment of triplets of ligand atoms on triplets of site points which are the centres of alpha sphere created in the potential binding sites. The interaction between various proteins such as p53, bax, bcl-2 and caspase 3 protein and ITCs are hydrogen bond formation, hydrophobic and polar interaction between aminoacid and the carbon and nitrogen atom of the isothiocyanate. The isothiocyanates such as SFN and I3C got anchored in p53 protein by forming a hydrogen bond with various aminoacids such as THR 6 ILE 9 GLU 16,20 GLN17 and O1 (3) with GLN 8,17 LEU 25 ARG 10,19,14 and ASP 28,29. In bax O1 (3) forma a hydrogen bond with LEU 63 whereas N1 (4) with GLU 163.in bcl-2 proteins. Caspases are synthesized as relatively inactive zymogens that become activated by scaffold-mediated transactivation or by cleavage via upstream proteases in an intracellular cascade [358]. Caspases are synthesized as relatively inactive zymogens that become activated by scaffold-mediated transactivation or by cleavage via upstream proteases in an intracellular cascade. Caspases (cysteine aspartatic acid proteases) play an essential role at various stages of the apoptotic process. Caspase zymogens possess an N-terminal prodomain and a linker peptide within the protease domain, which are cleaved to render
an active caspase. There exists a polar interaction between ASP 253 with O1 (3) and H1 cation interference between ITCs and caspases. The various hydrogen bonding and hydrophobic interactions reported were responsible for their binding and their energy of binding were found to be closer with 5 Fluorouracil (positive inducer) of apoptosis. Frequency of occurrence for all the proteins seems to fairly appreciable when compared with the 5FU.
Figure 7.1 Mechanism of Sulforaphane binding with various apoptosis proteins a) p53 (tumor suppressor) bonding with SFN b) Bax (proapoptotic) protein with SFN c) Bcl-2 (antiapoptotic) protein with SFN d) caspase 3 (Cysteine protease) with SFN as shown in JMol viewer (in built visualisation software of Docking server)
Figure 7.2 Visualisation of lowest energy conformation of binding of SFN with various apoptosis proteins a) p53 with SFN b) Bax with SFN c) bcl-2 with SFN d) Casp-3 with SFN as shown in ASTEX viewer (default viewer of Docking server)
Table 7.1 List of various parameters involved in the binding of isothiocyanates with apoptotic proteins

<table>
<thead>
<tr>
<th>Parameters</th>
<th>p53</th>
<th>Bax</th>
<th>bcl-2</th>
<th>casp-3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SFN</td>
<td>SFN</td>
<td>SFN</td>
<td>SFN</td>
</tr>
<tr>
<td>Est Free energy of Binding (kcal/mol)</td>
<td>-2.73</td>
<td>-3.8</td>
<td>-3.19</td>
<td>-4.36</td>
</tr>
<tr>
<td>Est inhibition Constant, Ki</td>
<td>6</td>
<td>635.99</td>
<td>521.7</td>
<td>164.7</td>
</tr>
<tr>
<td>vDW + Hbond+ dissolve Energy (kcal/mol)</td>
<td>-4.74</td>
<td>-3.64</td>
<td>-3.69</td>
<td>-6.41</td>
</tr>
<tr>
<td>Electrostatic Energy (kcal/mol)</td>
<td>-0.03</td>
<td>-0.16</td>
<td>-0.1</td>
<td>-0.05</td>
</tr>
<tr>
<td>Total intermol Energy (kcal/mol)</td>
<td>-4.76</td>
<td>-3.8</td>
<td>-3.8</td>
<td>6.46</td>
</tr>
<tr>
<td>Frequency (%)</td>
<td>18</td>
<td>18</td>
<td>23</td>
<td>33</td>
</tr>
<tr>
<td>Interaction Surface</td>
<td>396.56</td>
<td>250.65</td>
<td>343.347</td>
<td>381.6</td>
</tr>
</tbody>
</table>
7.4 Discussion

7.4.1 Docking of various apoptotic proteins with Isothiocyanates using Autodock

Bioinformatics is seen as an emerging field to study the protein–protein interactions (PPIs) of all biological processes such as oncogenesis [359]. SFN interacted with p53 that promotes sequestration of p53 in the cytoplasm, thereby inhibiting its nuclear activity and produces conformational changes thereby initiating the caspase cascade. Protein of p53, the “guardian of the genome,” is a major player in cell cycle arrest and apoptosis in response to the diverse endogenous and exogenous stress signals [360]. The p53 is a tumor suppressor that is essential for the prevention of cancer development. Loss of p53 function is one of the early events in immortalization of human cells. Another function of p53, independent to its nuclear localization, is to regulate mitochondrial membrane potential by interactions with the mitochondrial proteins bcl-2 and bax. Sampling receptor flexibility is challenging for database docking thus analysing multiple flexible regions of the binding site independently, recombining them to generate different discrete conformations. Flexible-receptor docking methods can predict more accurate binding geometries of ligands than rigid-receptor docking, few have been tested in database screening application [361]. The various parameters studied in this study are Free energy of binding, inhibition constant, intermolecular energy. Binding between the receptor and ligand is the sum of various parameters such as intermolecular energy+ Vander waals energy +hydrogen bond energy+electrostatic energy- unbound energy. The other parameters such as inhibition constant and intermolecular energy is directly proportional to binding of receptor and the ligand. Negative energy of binding indicstes stable system of combination. An important issue is how receptor flexibility affects the ability of a docking method to distinguish ligands from a much larger list of “decoy” molecules in these screens. This is the typical situation when docking a diverse database of several hundred thousand molecules, almost all of which would not be expected to bind to a particular target. This parameter can determine the specificity and activity of ligands in apoptosis induction. The structure activity relationship of various apoptosis proteins with ITCs proved their stability by studying various interactions involved in the binding. This study may provide an insight to the structural and molecular basis of their interaction in insilico level.