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In Silico Interaction Studies of H-RNR with Gemcitabine: A Tool for Anti-Tumor Drug Development

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Abstract

Background: Ribonucleotide Reductase (RNR) is an enzyme responsible for the reduction of ribonucleotides to their corresponding Deoxyribonucleotides (DNA), which is a building block for DNA replication and repair mechanisms. The key role of Ribonucleotide Reductase in DNA synthesis and control in cell growth has made this an important target for anticancer therapy. Increased RNR activity has been associated with malignant transformation and tumor cell growth. Gemcitabine, an metabolite (chemotherapy drug), which prevents the formation of DNA and RNA of the cell by interfering with the nucleic acid formation, which in turn ceases cell replication and causes cell death.

Results: The present investigation aims in using the X-Ray crystallography structure of human RNR for performing docking studies with Gemcitabine. The docked inhibitor Gemcitabine binds at the active sites GLU169, SER200 and ASN289. The inhibitor Gemcitabine suppresses the growth of tumors and is a type of chemotherapy, which interfere with the growth of rapidly growing cancer cells and eventually causes cell death. It is used alone or in combination with other chemotherapic agents. The present study was designated to evaluate the efficacy of binding pockets present in Gemcitabine pooled with Ribonucleotide Reductase.

Conclusion: We demonstrated that insilico docking studies was successfully carried out to identify the best binding site to inhibit the activity of RNR using the inhibitor Gemicitabine. As a conclusion, we could prove that the sites GLU 169, SER 200 could be the best possible sites where Gemcitabine

could inhibit RNR activity. This study helps to understanding the sites that prove to promote oncogenic activity along with the purposeful aspects and also aids in the progression of novel inhibitors for the human RNR.

Keywords: Ribonucleotide reductase (RNR), Gemcitabine, Active sites, Chemotherapy.

Introduction

Ribonucleotide Reductase (RNR) is an essential enzyme in the cell, responsible for converting ribonucleotides into deoxyribonucleotides,hence plays amajor role in central dogma of protein synthesis (Lewis et al 1978; Reichard 1993; Wright et al 1990), and also repair DNA in all living cells (Eklundet al. 2001). In mammalian cells, this enzyme contains two dissimilar protein components, R1 and R2, which are encoded by two different genes located on different chromosomes (Cocking et al 1987). Protein R1 is a homodimeric structure, with a molecular mass of 168kDa, and has substrate and allosteric effector sites that control enzyme activity and substrate specificity (Wright, et al 1990). Protein R2 is a homodimer, with a molecular mass of 88kDa, And forms two equivalent dinuclear iron centers that stabilize a tyrosyl free radical, required for the initiation of electron transformation during catalysis. R1 and R2 proteins interact at their C-terminal ends to form an active holoenzyme (Wright, et al 1990).hence the above discussion plays a major role in combating cancer ,thereby it can be called as the potential target of malignant cancer. Certain major consideration which makes it an excellent target includes substrate binding site and allosteric binding site along with its substrate specificity.

An increased interest in RNR as a target for cancer therapy is seen ever since the human ribonucleotide reductase of a new type was identified to be regulated by p53. The p53 actively suppresses tumor formation but on mutation several forms of cancer are developed. As much as over 80% of the human tumors have been found to contain mutations in p53 or in the pathway that directly regulates it. Mammalian RNR-R2 is located in the cytoplasm and regulated by the cell cycle. The new R2 gene product is called p53R2 and found to be located in the nucleus. The p53 binds to a sequence in the first intron of p53R2 gene and is required for directly activating its transcription. It was reported recently that p53 enzyme binds both R2 and p53R2 subunits in testing cells but upon exposure to UV radiation, they dissociate from p53 and bind to R1. Perhaps the regulation of RNR activity by p53 is more complex than activation of P53R2 (Eklund, H et al. 2001)

Materials and Methods

Ligand preparation and Active site identification

The X-Ray crystallography structure of RNR was used for docking. The Gemcitabine, an antimetabolite is used as a ligand. (PubChem Compound ID: CID: 60750), were retrieved from NCBI PubChem Compound database. Ligand structure

is shown in fig1. AutoDock 4.0 was used for this docking study. The active site residues of the ligand Gemcitabine was identified using Qsite finder. (http://www.modelling.leeds.ac.uk/qsitefinder/).

Docking RNR with Gemcitabine using Autodock

The "AutoDock Tools" was used to prepare, run, and analyze the docking simulations. AutoDock requires pre-calculated grid maps, one for each atom type present in the flexible molecules being docked and its stores the potential energy arising from the interaction with rigid macromolecules. This grid must surround the region of interest in the rigid macromolecule. The grid box size was set at 70, 70 and 70 A° (x, y, and z) to include all the amino acid residues that present in rigid macromolecules. AutoGrid 4.0 Program, supplied with AutoDock 4.0 was used to produce grid maps. The spacing between grid points was 0.375 angstroms. The Lamarckian Genetic Algorithm (LGA) was chosen search for the best conformers. During the docking process, a maximum of 25 conformers was considered. The population size was set to 100 and the individuals were initialized randomly. Maximum number of energy evaluation was set to 500000, maximum number of generations 1000, maximum number of top individual that automatically survived set to 1, mutation rate of 0.02, crossover rate of 0.8, Step sizes were 0.2 Å for translations, 5.0° for quaternions and 5.0° for torsions. Cluster tolerance 0.5Aº, external grid energy 1000.0, max initial energy 0.0, max number of retries 10000 and 10 LGA runs were performed. All the AutoDock docking runs were performed in Intel(R) Xeon(R) CPU 5150 @2.66GHz, 1.98GB RAM, Apple system. AutoDock was compiled and run under Windows XP operating system.

Docking RNR with Gemcitabine using Biosuite

Docking aids in finding favourable binding configurations between a flexible ligand and a macromolecular target (usually a protein molecule). Identification of the ligand poses in 3D space when it binds to a target using Docking. Using the functionalities provided in the Drug Design module, one can identify lead-like molecules from a set of molecules, redesign them and predict their activities. Thus, lead optimization can be achieved iteratively. If the target structure is known, then the lead optimization can be done using the structure based method, such as by docking.

Docking is performed using Docking module under Drug Design module in Biosuite package. Genetic algorithms (Morris et al (1998)) are used in Biosuite 3.0 for docking. Through a number of runs of the GA cycle, a conformation having minimum energy is obtained. Active site selection is performed using PASS. Conformation search functionality generates the conformations for an input molecule, clusters the conformations and displays energy and torsion angle values of low energy conformations. All the Biosuite docking analysis is performed using Intel(R) Core(TM)2 CPU 6300 @ 1.86 GHz. Biosuite 3.0 was compiled and run in Linux operating system (Fedora core 8).

Results and Discussion

The sequence of RNR (P31350) with 389 residues was retrieved from swiss-prot database (http://www.expasy.ch/sprot/). BLASTp (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi) was carried out against the Protein Data Bank (PDB) in order to find suitable templates for modeling of human RNR. The protein 1H0N which is an OXIDOREDUCTASE (COBALT SUBSTITUTION OF MOUSE R2 RIBONUCLEOTIDE REDUCTASE TO MODEL THE REACTIVE DIFERROUS STATE) of Mus musculus is selected as template. The template structure has 390 residues and has 91% identity with the target sequence. The E-Value and score is found to be 0 and 744respectively.

The target sequence was modeled using Discovery Studio. The sequences of targets and templates structures have been aligned and models were built using Modeller Program. The energy of the target protein was minimized in Steepest Descent followed by Conjugate Gradient method. Each of the minimization methods were carried out with CHARMm force field. Atomic clashes (bumps) were removed by rotating side chain torsion angles using What if web server. Again energy minimisation was carried out and the value is found to be -18908.04063 kcal/mol. Ramachandran plot is visualized in Discovery Studio of which 5 residues (GLU 305, GLY294, GLY304, GLY251, GLY 273) were found in the disallowed region, as shown in fig3. Structural validation is done using Structural Analysis and Verification Server. Residues in the most favoured region are found to be 94.3%.

After inspecting the modeled HRNR structure, properties of the ligand (Ligand structure is shown in fig1.) is inspected using Lipinski rule. The rule states, that most "drug- like" molecules have logP <= 5, molecular weight <= 500, number of hydrogen bond acceptors <= 10, and number of hydrogen bond donors <= 5. Molecules violating more than one of these rules may have problems with bioavailability. The rule is called "Rule of 5", because the border values are 5, 500, 2*5, and 5. It is tested using Molinspiration tool. (http://www.molinspiration.com).

The molecular properties of Gemcitabine are listed in table 1. These properties satisfy the Lipinski rule. Hence Gemcitabine has drug like properties and is also bio-available.

Table 2: Molecular Properties of Gemcitabine.

Property	Description	Value
logP	Octanol-water partition coefficient	-1.603
TPSA	Polar surface area	110.61
Natoms	Number of non hydrogen atoms	18
MW	Molecular weight	263.2
nON	Number of hydrogen-bond acceptors (O and N atoms)	7
nOHNH	Number of hydrogen-bond donors (OH and NH groups)	4
nviolations	Number of Rule of 5 violations	0
nrotb	Number of rotatable bonds	2
volume	Molecular volume	203.356

Table 3: Molinspiration drug-likeliness score.

Properties	Score
GPCR ligand	-0.09
Ion channel modulator	-1.22
Kinase inhibitor	0.02
Nuclear receptor ligand	-1.96

Prediction of bioactivity score for the most important drug targets (GPCR ligands, kinase inhibitors, ion channel modulators, nuclear receptors) and possible molecular toxicity is listed in this table.

The activity of the inhibitor Gemcitabine has been analyzed by means of docking studies using Autodock and Biosuite. As a first step in docking studies, active site of the RNR is found using Q-site finder. GLU 169, SER 200 and ASN 289 were found to be the best active sites of the modeled H-RNR.

H-RNR, the Macromolecule and Gemcitabine, the ligand is used for rigid docking. Grid parameters were set as mentioned earlier and spacing between grid points was 0.375 angstroms. Genetic algorithm was chosen for conformers. During the docking process, a maximum of 25 conformers was considered. After the simulations were complete, the docked structure is analysed and the interactions were seen. Hydrogen bond interactions and binding distances were measured for the best conformers. Cluster Rank 4 and subrank 1 with binding energy -4.77 kcal/mol at 6^{th} run has two hydrogen bond interactions at residues SER200, ASN289 with Cluster RMSD 0.0 and reference RMSD 87.17. The hydrogen bond distance between OD1 atom of ASN289 and hydrogen atom of the ligand is found to be 1.918. The hydrogen bond distance between HG atom of SER200 and oxygen atom of the ligand is found to be 1.814. Docked image is shown in Fig. 1b and hydrogen bond interaction was performed. Cluster Rank 8 and subrank 1 with binding energy -3.98 kcal/mol at 25^{th} run has one hydrogen bond interaction at residue GLU169 with Cluster RMSD 0.0 and Reference RMSD 90.24. The hydrogen bond distance between Oxygen atom of GLU169 and hydrogen atom of the ligand is found to be 1.968. Docked image is shown in Fig. 1c .

Docking of H-RNR with Gemcitabine in Biosuite 3.0 using Genetic Algorithm has generated 25 solutions of which solution2 had best interaction. Solution 2 (Cluster Id 1) has binding energy -12.83 kCal/mol with RMSD 0.0. RMS tolerance was found to be 0.5. Interacting residues with hydrogen bonding were GLU169 and SER 200. Donor acceptor distance, Hydrogen acceptor distance and other angles were obtained. Donor acceptor distance between GLU169 and ligand hydrogen, SER200 and ligand hydrogen was found to be 2.49, 2.58 respectively.

Conclusion

Molecular docking simulation study was undertaken to investigate the binding

mechanism of gemcitabine to inhibit human RNR. From these docking studies, we conclude that H-RNR activity could possibly be inhibited by Gemcitabine. GLU169, SER200 and ASN289 could be the best possible sites where Gemcitabine can bind to the H-RNR and aid in the control of tumour cell growth. This study helps us to understand the functional aspects of the ligand and HRNR and also aids in the development of novel inhibitors for the human RNR. Finally, we wish to conclude that this work can be considered as the preliminary approach in determining the functional characteristics of Human Ribonucleotide Reductase and the ligand Gemcitabine. Similar work could be done using other new molecules which could interact with this receptor.

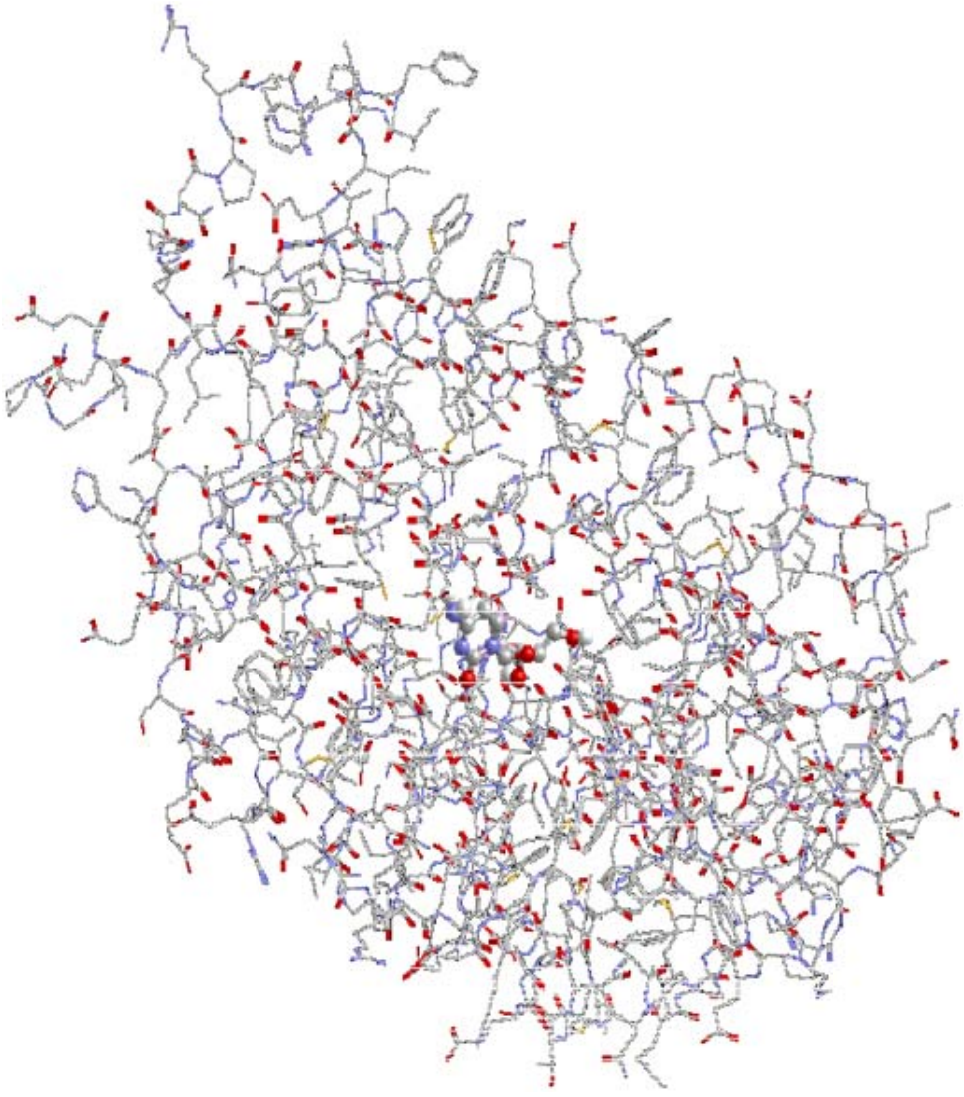

Figure 1a: Docked Image of H-RNR with Gemcitabine using Biosuite 3.0. Binding of Gemcitabine with HRNR at the active sites GLU169 and SER200 of HRNR with hydrogen atom of the ligand using Biosuite 3.0.

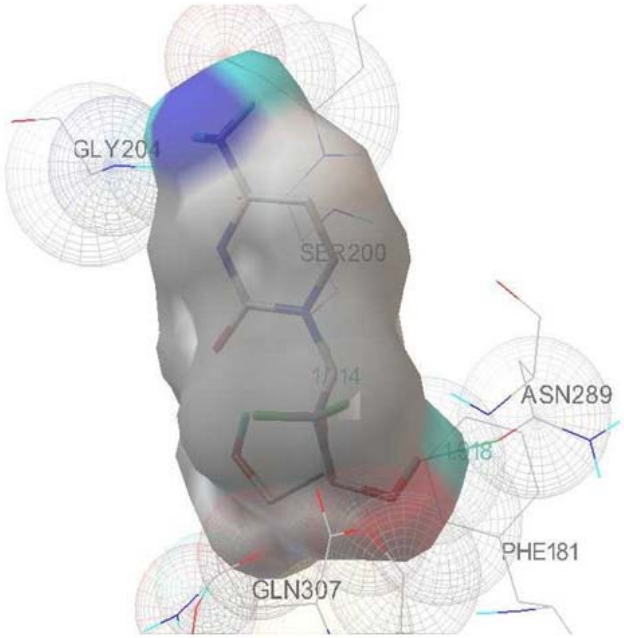

Figure 1b: Docked Image of H-RNR with Gemcitabine (6th conformation) using Autodock4. (SER200 of Human RNR forms hydrogen bond with oxygen atom of the ligand with a distance of 1.814Å. ASN289 of Human RNR forms hydrogen bond with hydrogen atom of the ligand with a distance of 1.918Å.).

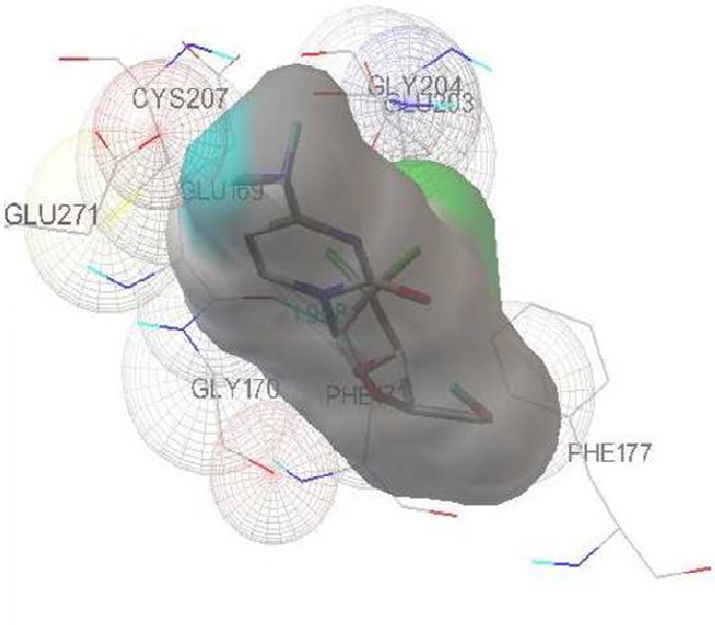

Figure 1c: Docked Image of Human RNR with Gemcitabine (25th conformation) using Autodock4. (GLU169 of H- RNR forms hydrogen bond with hydrogen atom of the ligand with a distance of 1.968Å.)

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