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Computational Simulation of Mitoxantrone Binding with Human Serum Albumin

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Abstract

Mitoxantrone (MTX) is a clinically used antitumor anthracycline, which is transported to the target tissues by human serum albumin (HSA). Being less toxic unlike other member of this family, its binding characteristics are therefore of immense interest. The protein and the ligand were prepared with the aid of CORINA, protonated with insight II and best conformation was sought by employing Gold V. By docking procedure, site III has been assigned to posses the binding site for MTX with the binding affinity (Ka) = 1.58 x 106 mol-1. Molecular docking calculations placed MTX at digitoxin binding site of HSA. The interaction was found to be thermodynamically favourable (ÄG° = -35.53 *K*Jmol-1). Further analysis of the MTX binding site on to the HSA suggested that the type of interactions that contribute in this binding are hydrophobic contacts, hydrogen bonding and electrostatic interactions. This study presents binding mechanism in a unified way that is simple, yet stringent, more straightforward, more reliable and informative.

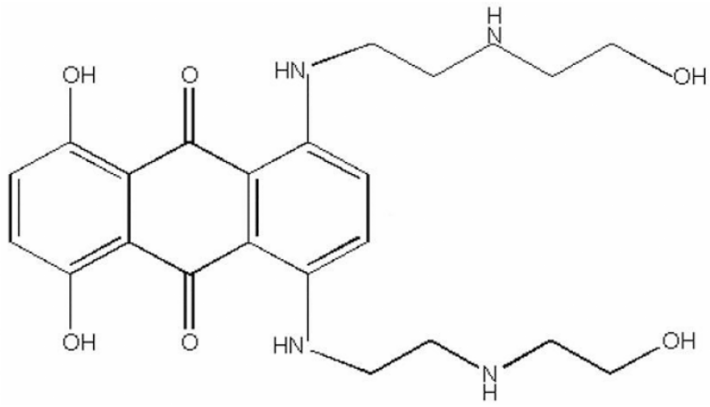
Key words: Human serum albumin; Mitoxantrone; Drug binding; Molecular docking

Introduction

Mitoxantrone (MTX) an analogue of the anthracycline antibiotics belongs to the anthracenediones, a class of synthetic chemotherapeutic agents. It has shown significant clinical effectiveness in the treatment of a range of human malignancies, mostly metastatic breast cancer, acute myeloid leukemia and nonhodgkins lymphoma. (Hagemeister *et al.*, 2005; Tsavaris *et al.*, 2005). MTX is also used to treat multiple sclerosis (Buttinelli *et al.*, 2007). In contrast to other anthracyclines, mitoxantrone produces less side-effect such as nausea, vomiting, alopecia and cardiac toxicity (Cornbleet *et al.*, 1984; Neidhart *et al.*, 1984), which bring its wide applicability. Human serum albumin is the principal extracellular protein as it is responsible for transporting many exogenous and endogenous substances, including many drugs with a relative constant level of 3.5-4.5% (w/v) (Quevedo *et al.*, 2001). Crystallographic analyses revealed that HSA is a single polypeptide chain of 585 amino acids with a largely-helical (67%) triple-domain structure that assemble to form a heart-shaped molecule. The protein contains three homologous á-helix domains (I–III) (Carter *et al.*, 1994). Serum albumin has two well-known ligand binding sites, site I and site II. Site I is located within subdomain II A and is known as azapropazone warfarin site (Fehske *et al.*, 1981). On the other hand, site II, also known as the indole-benzodiazepine site, is located within subdomain III A (Rahim *et al.*, 1995). Site III and IV specific for the binding of tamoxifen and digitoxin, respectively (Ojingwa *et al.*, 1994). Binding of a drug to HSA results in an increased solubility of drugs in plasma their decreased toxicity, and /or protection against oxidation of the bound ligand. Binding can also have a significant impact on the pharmacokinetics of drugs, e.g. prolonging in vivo half life of the therapeutic agent. However, too strong binding prevents drug release in tissues. That is why HSA binding information is one of the key characteristics of a drug determining its ADME properties. Co-binding of two drugs or displacement of one drug by another may alter the therapeutic drug level and can lead to serious health conditions. Drug distribution within the body is determined mainly by free (unbound) concentration of drug in circulating plasma (Buxton *et al.*, 2005). The unbound fraction, in turn, depends on drug absorption by plasma proteins. Human Serum Albumin (HSA) is the

most abundant blood plasma protein and is produced in the liver. It has been shown to shuttle a broad range of endogenous and exogenous ligands, including more than 70% of drugs (Kratochwil *et al.*, 2002). A high binding affinity for protein has been observed for drugs possessing acidic or strong electronegative functional groups (Groth *et al.*, 1972), which can bound to more than one binding site with different specificity. Thus, detailed knowledge of the binding interaction of a drug on albumin and of their relative strengths is important especially for the drugs given in combination regimes. Due to the difficulties for measuring the bound fraction of drugs by means of *in vivo* methods and inconsistency of different *in vitro* techniques, such as ultrafiltration, equilibrium dialysis and gel filtration, but results using these methods can differ significantly. For example, studying the binding of fleroxacin by means of equilibrium dialysis showed that 23% of the drug was bound, while the same study carried out using the ultrafiltration method reported it as 47% (Brunt *et al.*, 1990) opens the door for reliability on insilico methods. Among this molecular docking procedure is best suited. This not only save time and labour but also give results with accuracy upto amino acid residue involved in the binding pocket ,which would not be possible in solution studies. In present report we demonstrate successful prediction of binding activity to HSA among the number of existing drug binding sites and evaluated the binding mode on the basis on amino acid involved.

Figure 1



Materials and Methods

Genetic Algorithm

Genetic algorithm (GA) is a computer program that mimics the process of evolution by manipulating a collection of datastructures evolution by manipulating a collection of datastructures called chromosomes. Each of these encodes a possible solution (in terms of a possible ligand-receptor interaction) to the docking problem and may be assigned fitness score based on the relative merit of that solution. The GA utilizes a novel representation of the docking process. Each chromosome encodes an internal conformation and protein active site, and includes a mapping from hydrogen-bonding sites in the ligand and protein. On decoding a chromosome, least-squares fitting process is employed to position the ligand within the active site of the protein in such a way that as many of the hydrogen bonds suggested by the mapping are formed. The fitness of a decoded chromosome is then a combination of the number and strength of the hydrogen bonds that have been formed in this way and of the van der Waals energy of the bound complex.

Preparation of the Protein and the Ligand

Known crystal structures of Human Serum Albumin (HSA) (PDB Id: 1h9z) was obtained from the Brookhaven Protein Data Bank. The two dimentional (2D) structure of Mitoxantrone (Fig.1) was downloaded from Pubchem (pubchem.ncbi.nlm.nih.gov). 2D to three dimensional (3D) conversion was done with CORINA (www.mol-net.de). Water molecules and ions were removed (including ordered water molecules) and hydrogen atoms added at appropriate geometry groups within the protein were ionized as required at physiological pH. The structure of HSA was protonated in InsightII (www.accelrys.com). Genetic algorithm was implemented in GOLDv3.1.1 that was applied to calculate the possible conformations of the drug that binds to the protein. The genetic algorithm parameters used are: Population size-100; Number of Islands-5; Niche size-2; Selection pressure-1.1; Migrate- 2; Number of operators-100,000; Mutate-95; Crossover- 95. During docking process, a maximum of 10 different conformations was considered for the drug. The conformer with the lowest binding free energy was used for further analysis. Two dimensional structure of Mitoxantrone downloaded from PubChem (pubchem.ncbi.nlm.nih.gov).

Molecular Docking Simulations

All the conformations were then evaluated by X-SCORE. RMSD values of the best scored conformations of these proteinligand complexes for the reported four binding site were evaluated. The binding energy of docked complexes was calculated using X-Score (Wang *et al.*, 2002). The scor-

ing functions have all the necessary elements that correspond to the non-covalent interactions in a conventional force field, such as the van der Waals interaction and the electrostatic interaction. Besides that, it also considers the hydrophobic effect and thus provides a better estimation of binding free energies. A special feature is that three different algorithms have been implemented to calculate the hydrophobic effect term, which results in three parallel scoring functions. All three scoring functions are calibrated through multivariate regression analysis of protein-ligand complexes, which reproduce the binding free energies of the entire. These three scoring functions are further combined into a consensus scoring function, X-SCORE. The residues that are making hydrogen bonding and hydrophobic interactions were calculated using Getneares, which is a tool available with DOCKv5.1.1 (Kuntz *et al*., 1982).

Results and Discussion

Drug Protein Interaction

The complementary applications of molecule modeling have been employed by computer methods to improve the understanding of the interaction of mitoxantrone and HSA. The best docking result is shown in figure 2. As predicted from the docking procedure the MTX binding would occur at site III on HSA. The binding affinity (*K*a) was found to be 1.58 x 106 and predicted binding energy (ÄG°) of MTX to HSA is -35.53 kJ/mol. Negative value of ÄG° shows that the binding reaction is thermodynamically favourable and the value of binding constant predicts high binding affinity between protein and the drug.

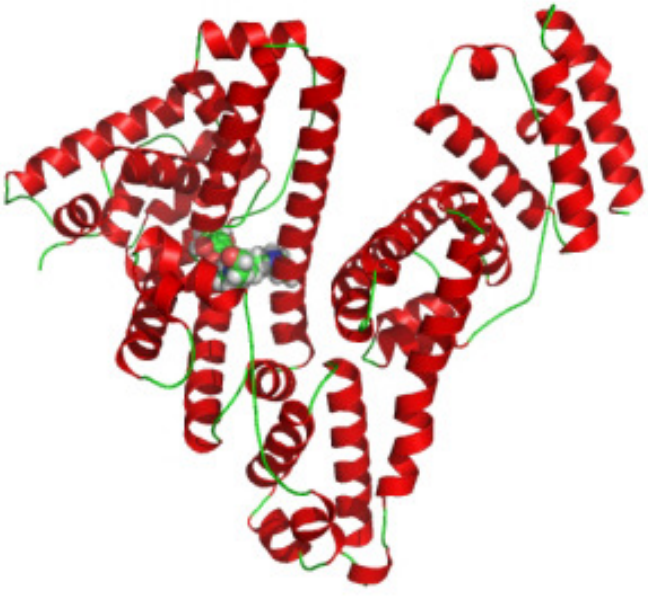
Binding Site

The binding site of MTX was found to be on Domain III of HSA. This site is described as site III or digitoxin binding site. Amino acid residues involved in the binding of MTX to HSA were predicted and there respective molecular distances from the bound drug have been evaluated with the Getnears as presented in Table 1. The present data revealed that Tyr150 is the closest residue to be found in the vicinity (5 Å) of drug molecule, whereas, Ser192 was found to be the farthest. The close proximity of tyrosine to ligand suggest that fluorimetric techniques to be a sensitive tool to probe this interaction for in solution studies.

Binding Mode

The validation of the binding mode as per the amino acid residue predicted to be the part of the binding site in Figure 2. Where Phe149, Ala151, Ala201, Gly248, Tyr148, Tyr150 can make hydrophobic with phenol ring of MTX. The interaction between MTX and HSA is not exclusively hydrophobic in nature since there are several ionic (His242, His247, Arg197, Arg257, Lys106) as well as polar residues (Leu250, Ser192, Glu153, Pro147, Pro152, Cys200, Cys245, Cys253, Cys246, Gln196) in the proximity of the bound ligand (within 5 °A).These polar residues probably play an important role in stabilizing MTX via Hbonds and electrostatic interactions. Basic amino acids like Arg, His and Lys in the vicinity of MTX can be the targets for interactions with negatively charged carbonyl oxygen functions of the dihydroxyanthraquinone moiety. The hydrogen-bonding or electrostatic interaction acts as an “anchor”, intensely determining the three dimensional position of MTX in the binding pocket and facilitating the hydrophobic interaction of the dihydroxyanthraquinone rings with the side chain of protein.

Figure 2



The MTX binding properties of HSA was mapped by molecular docking. Docking calculations found MTX to be best located at the digitoxin binding site on HSA. The model showed the microenvironment of MTX to be rich in polar (basic) amino acid residues able to stabilize the ligand. This work provides comprehensive insight and understanding of the molecular interaction of MTX with HSA *in vivo*. These results also indicate the reproducibility and accuracy of the computational studies. The major source of fluctuations

arises from conformational uncertainty in model building. Also, the lack of understanding of chemical effects and ability to model them, e.g. the protonation of atoms is a vexing cause of discrepancy between the calculated and experimental studies.

Mitoxantrone docked onto Human serum albumin to show its binding site on domain III. MTX, depicted in space fill model (light green), and HSA, represented in ribbon struture (red). The image was made using Pymol (pymol.sourceforge.net).

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