3D Computational Modeling of Human P-gp NBD2 with Papyriferic Acid Derivatives

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Abstract

Human P-gp is one of the protein responsible for the multidrug resistance (MDR) development. MDR is a major cause of the cancer chemotherapy. In this paper, we performed homology modeling, docking study of papayriferic acid into the P-gp nucleotide binding domain (NBD2). For human P-gp, X-ray crystal structure is not known yet. We developed homology model for human NBD2 using HlyB ABC transporter structure (PDB code: 1XEF, resolution 2.5 Å). Docking study was performed using Autodock. Docking result was analyzed, which shows that ligand docks into steroid binding site and interacts through hydrophobic and hydrophilic interactions.

Key words : P-gp, NBD2, Papyriferic Acid, Docking

1. Introduction

P-gp is a major protein responsible for the MDR. It belongs to ATP binding cassettes sub family B member 1 (ABCB1), is a transmembrane protein^[1]. P-gp expressed in kidney, liver, lung, intestine and blood brain barrier (BBB)^[2]. P-gp holds high clinical importance because it exports drug, substrates and chemotherapeutic agents, which results in lower intracellular concentrations of it^[3]. Moreover, unavailability of human P-gp X-ray crystal structure generated strong interest among researcher to know the mechanism of action of drug, substrate and modulators. P-gp is the most studied protein among ABC transporter^[4].

Human P-gp consists of 1280 amino acids (UniProt KB: P08183), with the molecular weight of 170 kDa. P-gp consists of two transmembrane domains (TMD) and two nucleotide binding domain (NBD). TMDs are located in the cell membrane, whereas NBDs are located into the cells. Each TMD consists of six helices connected with the NBDs through intracellular linker (ICL). Half transporter consists of TMD+NBD (610 amino acids) and linker consists of 60 amino acids.

NBDs consists of ATP binding sites, ATP hydrolysis generate energy which subsequently utilize to transport the xenobiotics from cell^[5].

Verapamil and Cyclosporin are the first generation P-gp inhibitors, but removed from chemotherapy because of high dose related higher toxicity. The second generation of inhibitors consists of dexamethasone and PSC833 were failed to show ideal drug property and removed from chemotherapy because of serious drug-drug interactions. The third generations of inhibitors are synthetic one and devoid of any structural similarity with previous one. Although they possess high efficacy, increased selectivity and low toxicity. Tariquidar (XR9576), elacridar (GF120918) zosuquidar (LY335979) and laniquidar (R1010933) are representatives of third generation modulators. Thus, to date the search for new non-toxic, potent modulator lacking pharmacokinetic interaction is still in progress. Recently, papyriferic acid derivatives have been shown to revert MDR in cancer cells^[6]. To show the binding mode of papyriferic acid derivatives into P-gp NBD2, a homology model was developed and docking study was performed.

2. Experimental Section

2.1. Sequence Alignment and Homology Modeling of P-gp NBD2

A FASTA sequence of human P-gp was retrieved

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(www.expasy.org; UniProt KB:P08183). The NBD2 sequence (1035-1273) was truncated and protein Blast (<http://blast.ncbi.nlm.nih.gov/>) was performed to identify suitable template for homology model. Blast search retrieved number of templates, but we chosen E-coli ABC transporter (PDB code 1XEF, resolution 2.5 Å) structure. NBD2 showed 48% sequence identity with 1XEF. After identification of suitable template, multiple sequence alignment was performed with that of the ClustalW 2.0 program (<http://www.ebi.ac.uk/Tools/msa/clustalw2/>). Aligned sequences of target and template were imported to Modeller9v4 program^[7] to generate homology model. With the given sequence alignment 100 homology models were generated for the NBD2 using modeller.

2.2. Molecular Docking of Papyriferic Acid Derivatives into NBD2 Model

To know the ligand interactions with the NBD2, a molecular docking study was performed with the two inhibitors. The most potent (**37**, $IC_{50}=1.3\ \mu M$) and medium potent (**29**, $IC_{50}=5.6\ \mu M$) papyriferic acid derivatives were chosen for the docking study. **37** and **29** were drawn and energy minimized using SYBYL 8.1 software package.^[8] Gasteiger-Hückel partial charges were assigned to **37** and **29** after minimization. NBD2 model was prepared for docking using Autodock Tool. Gasteiger-Hückel partial charges were assigned to NBD2, each atom were assigned using ADT atom type and non polar hydrogens were merged. Actual docking study was performed with Autodock4 software.^[9] Bind-

ing pocket for the inhibitors were assigned at the steroid binding site. The center of grid was assigned at the C α atom of the V1053 with $50\times 50\times 40$ grid points in XYZ direction. The grid spacing was 0.375 Å in each dimension. The global optimization was started with a population of 100 randomly positioned individuals, with a maximum of 2.5×10^6 energy evaluations, and a maximum of 2.7×10^5 generations. During each docking experiment, 30 poses were generated. The rest of the parameters were left to their default values. At the end of a docking experiment, a cluster analysis was carried out to get global minimum conformation of **37** and **29** in putative NBD2 cavity. Docking solutions with a all-atom root mean square deviation (RMSD) within 1 Å of each other were clustered together for **37** and **29** and ranked by the lowest docking energy.

3. Results and Discussion

3.1. Sequence and Homology Model Analysis

Aligned sequence of 1XEF and NBD2 is shown in Fig. 1. Modeller develops 100 models for NBD2 using template 1XEF. Final model for the analysis was selected based on the Procheck plot and one with the lower molecular probability density function score as well as with the lowest RMSD to template (1XEF) backbone. Ramachandran plot (Fig. 2) shows that the 98.7% residues are in most favorable and allowed region, whereas 1.4% residues are in generously allowed and 0.08% residues in disallowed regions. Careful scrutiny of disallowed regions residue showed

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1XEF  DITFRNIRFRY--KPDSPVILDNINLSIKQGEVIGIVGRSGSGKSTLTCLIQRFYIPENG 58
NBD2  -VTFGEVVFNYPTRPDIPVLQG-LSLEVKKGQTLALVGSSGCGKSTVVQLLERFYDPLAG 58
      **:  ::  *.  :** **:  .  :*.**:*:...: ** *.*****.:*:*** *  *

1XEF  QVLIDGHDALADPNWLRQVGVVLQDNVLLNRSIIDNISLANPG--MSVEKVIYAAKLA 116
NBD2  KVLLDGKEIKRLNVQWLRAHLGIVSQEPI LFDCSIAENIAYGDNSRVVSQEEIVRAAKEA 118
      **:***:::  :  :*** :*: *  :*:  ** :*:  .  .  :* *:::  *** *

1XEF  GAHDFISELREGYNTIVGEQGAGLSGGQRQRIAIARALVNNPKILIFDEATSALDYESEH 176
NBD2  NIHAFIESLPNKYSTKVGDKGTQLSGGQKQRIAIARALVRQPHILLDEATSALDTESEK 178
      .  *  **..*  :  *.  *  ***: *  :*****:*****.:*:***:*****  ***:

1XEF  VIMRNMHKICKGRTVIIIAARLSTVKNADRIIVMEKGKIVEQGHKELLLSEPELSYLY 236
NBD2  VVQALDKAREGRTCVIAHRLSTIQNADLIVVFQNGRVKEHGHQQLLAQKGIYFSMVS 238
      *:  .  :*.  :*** *:** *****:*** *:***:***:  *:*.**:***:  :*  :

1XEF  QLQSD 241
NBD2  V---- 239

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Fig. 1. Sequence alignment between template (1XEF) and target (NBD2).

Fig. 2. Ramachandran plot for selected NBD2 model.

that they are not belong to the binding pocket. NBD2 model aligned over 1XEF structure, which shows 0.283 Å RMSD for backbone. From the above analyses of model we concluded that our developed model is of good quality for the further computational study. The selected model was further used for the docking study of **37** and **29**.

3.2. Docking Analysis of **37** and **29** into NBD2 Cavity

To investigate the binding modes of **37** and **29** into NBD2 a docking study was performed with Autodock. Binding mode of **37** is shown in Fig. 3. Papyriferic acids are not steroid but their structure is very similar to steroid nucleus and it is obvious that they will share the same binding site as steroid. From Fig. 3, it is evident that the **37** docked into a steroid binding site and partly overlaps the ATP binding site. Higher hydrophobic interactions are measured with the active site residues of NBD2. However, strong hydrogen bond interactions were also observed. Hydroxyl group on C25 shows hydrogen bond with the Y1044, and two bonds with Q1080. The ester group oxygen at C12 posi-

tion hydrogen bonded with the T1078. Another hydrogen bond was observed between the carbonyl group of the G1073 and oxygen at C3 position of **37**. Hydrophobic interactions are observed with the I1050, P1051, V1052, Q1054 and main chain of P-loop containing residues G1073, C1074, G1075. Y1044 was found to interact through both the way as well hydrophobic interaction was observed with the Y1086.

Docked mode of compound **29** is shown in Fig. 4. 4 Å surrounding residues were shown for clarity. **29** showed only one (Q1248) hydrogen bond interaction and it mainly interacts through the lipophilic interactions. Strong lipophilic interactions were observed for the Y1044, I1050, P1051, V1052, and main chain of P-loop containing residues G1073, C1074 and G1075. S1071 and S1072 from P-loop also found to be interacting with the **29**.

Previous study of steroid showed that it binds in steroid binding site and overlap ATP binding site.^[10] However, our results are in line with the previous report that the inhibitors dock into the steroid binding site and overlap ATP binding site. The most potent papyriferic acid derivative **37** shows more number of hydrogen

Fig. 3. Binding mode of **37** into P-gp NBD2. Interacting residues in vicinity of 4 Å of **37** were shown in yellow stick, whereas **37** shown in cyan stick. Hydrogen bond contacts (Y1044, G1073, T1078 and Q1080) were shown in red dashed line.

Fig. 4. Binding mode of the **29** into NBD2. 4 residues surrounding **29** were shown for clarity.

bonds compare to medium active **29**. It is reported in SAR data that hydroxyl group at C25 position is very important for MDR reversing activity. For **37** we observed hydrogen bond for this hydroxyl group but it is absent in **29**, this might be the reason why compound **37** is more potent than the **29**. Also some structural differences like morpholino versus N(CH₃)₂ made substantial difference in activity. From Figs. 3 and 4 it is evident that the ligands orientations are different. In case of **37**, it could be seen that the OAc at C12 position is docked near the T1078 and hydrogen bonded with it. In contrast the same group in **29** placed near R1047. Moreover, in case of **37** the C3 substituent is docked near Q1054 and in **29** it binds near Q1248.

4. Conclusion

P-gp is a key protein in MDR. The structural modeling of it is necessary to identify the interaction mechanism of inhibitors. In present paper we performed homology modeling based on the closest identical structure. Two compounds were docked to know their interaction profile with the NBD2. It was observed that the both compounds interact through hydrophobic interaction and hydrophilic interaction. Compound **37** shows more number of hydrogen bonds and it was observed that the OAc group at C12 position is important for the reversing activity. Our preliminary computational model of inhibitor-NBD2 interactions could be helpful to understand mechanism of inhibition of P-gp.

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