

Structural Insight into the Binding Interactions of Native and Mutant Structures of Dihydrofolate Reductases

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ABSTRACT: Dihydrofolate reductase (DHFR) is an important enzyme in the cell and is the target for antifolate drugs such as methotrexate, pyrimethamine and trimethoprim. In this study, we compared the binding interactions of trimethoprim with native and mutant structure of DHFR by computational methods. We generated four mutant structures of DHFR by using UCSF chimera. Binding energies of trimethoprim with native and mutant structures of DHFR were computed by using Autodock. Finally, one mutant structure (1YHO_Q35I) which has been generated by computational methods, has shown a high affinity binding affinity towards the trimethoprim the native other mutant structures.

KEYWORDS: Affinity, Dihydrofolate reductase, Docking, Trimethoprim

1. INTRODUCTION

Dihydrofolate reductase, DHFR, is an enzyme that has a significant role in regulating the amount of Tetrahydrofolate in the cell [1]. It reduces dihydrofolic acid to tetrahydrofolic acid by using NADPH which can be converted to the Tetrahydrofolate cofactors. Trimethoprim is a bacteriostatic antibiotic and it inhibits the reduction of dihydrofolic acid (DHF) to tetrahydrofolic acid (THF). THF is an essential precursor in the thymidine synthesis pathway and interference with this pathway inhibits bacterial DNA synthesis. The mechanism of action of trimethoprim (TMP) is the inhibition of DHFR, an enzyme that participates in the recycling of folates by reducing dihydrofolate to Tetrahydro-folate in bacteria, pneumocystis and mammalian cells [2].

To carry out the computational analysis, the three-dimensional (3D) structures of native and mutant Dihydrofolate Reductase were taken from the crystal structures of Protein Data Bank [3,4]. Non-covalent molecular interaction between the face of an electron-rich π system and an adjacent cation were calculated for both the native and mutant structures by protein interaction calculator (PIC). We generated four mutant structures 1YHO_D21V, 1YHO_K18V, 1YHO_Q35I and 1YHO_R91Y by using UCSF chimera. Finally, binding energies of trimethoprim with the native and five mutants were calculated by autodock to identify the high affinity structure.

II. EXPERIMENTAL APPROACHES

2.1. Cation- π Interactions

Native and mutant structures of DHFR obtained from PDB data base. The cation-pi interactions of trimethoprim with native and mutant structures were calculated by Protein Interaction Calculator (PIC) server [5]

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2.2. Generation of mutants

The best mutating residues were selected on the basis of the results of CUPSAT server, whether they were stabilizing or destabilizing, on the basis of torsion angles and $\Delta\Delta G$ values. These residues were replaced with the potentially important ones, obtained from HotSpot Wizard, in UCSF Chimera 1.10.1 [6].

2.3. Molecular Docking:

Docking of native and mutant structures of DHFR and five model structures with trimethoprim ligand were performed using AUTODOCK version 4.2. [7, 8] Preparation of protein structures: Protein molecules were added to Molecular Graphics laboratory (MGL) Tools Version 1.5.6 and these tools were used to remove water molecules from these molecules and hydrogen's were also added with Kollman charges to the protein molecule. Finally all the files were saved in PDB format.

2.4. Ligand preparation: Trimethoprim molecule is taken from RCSB, protein data bank, with DrugBank ID-DB03125. The three dimensional structure was retrieved in SDF format after that UCSF Chimera was used to convert in inot PDB file.

2.5. Docking procedure: Protein and ligand molecules were loaded in Autodock tools win-dow and Grid box was built with respect to urea binding sites of that particular protein structures. Default parameters were set for all the docking. The resultant Grid files were saved in .gpf file format. Docking file was prepared with the help of loaded molecules in .dpf file format. Autogrid4.exe file was used for preparing grid log file and map files. Autodock4.exe file was used for preparing docking log file. Resultant (protein-TMP complex) files were saved in PDB format for further analysis.

Table.1.Cation-pi interactions

Donor Residue in Native type (PDB code-1YHO)	Acceptor Residue in Native type (PDB code-1YHO)	Distance (Å°)	Angle (in Degrees)	Donor Residue in Native type (PDB code-3S3V)	Acceptor Residue in Native type (PDB code-3S3V)	Distance (Å°)	Angle (in Degrees)
PHE34	ARG70	5.90	95.18	TYR33	ARG36	5.48	110.07
TRP57	ARG65	3.78	31.81	PHE34	LYS35	4.82	94.28
PHE58	LYS54	3.94	166.69	TRP57	ARG65	5.73	51.16
TYR162	LYS132	5.75	56.11	PHE58	ARG65	5.26	59.37
-	-	-	-	PHE64	LYS63	5.07	136.08
-	-	-	-	TYR156	LYS155	5.88	61.50
-	-	-	-	TYR162	LYS132	5.18	109.74
-	-	-	-	TYR182	ARG137	5.82	115.34

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III. RESULTS AND DISCUSSION

3.1.Cation-Pi interactions

Cation-Pi interactions of TPM-DHFR complexes were calculated by Protein Interac-tions Calculator (PIC) is a server. Naitve and mutant structures of DHFR which are avi-able in pdb database were chosen for this study. Distance between the two aminoacids and angles were computed for both the structure and values are compared in Table.1.a total of four Cation-Pi interactions were observed in native DHFR-TMP complex. Among four TRP57 –ARG65 is shortest distance Cation-Pi interaction with a distance of 3.75 A° and PHE34-ARG70 is the longest distance with a distance of 5.9 A°.PHE-LYS132 is found to be highest bond angle cation-Pi interaction with a bond angle of 166.69° and TRP57-ARG65 found to be smallest bond angle cation-Pi interaction with a bond angle of 31.81°. Eight Cation-Pi interactions were observed in mutant DHFR-TMP complex. PHE34 –LYS35 is shortest distance Cation-Pi interaction with a dis-tance of 4.82 A° and TYR156-LYS155 is the longest distance with a distance of 5.9 A°. PHE64-LYS63 is found to be highest bond angle cation-Pi interaction with a bond angle of 136.08° and TRP57-ARG65 found to be smallest bond angle cation-Pi interac-tion with a bond angle of 51.16°. in conclusion, mutant structure with TMP complex has shown comparatively better Cation-Pi interactions than native structure with TMP complex.

Generation of mutants

In our previous study, the following residues were obtained as potentially important sites for mutagenesis Pro26, Asp21, Arg91, Lys18, Gln35 with a mutability score of 9, 9, 9, 6 and 6. These residues were then checked for stabilizing and destabilizing alter-natives in the CUPSAT server and the following results were obtained on the basis of of torsion angles and $\Delta\Delta G$ values. On the basis of torsion angle’s stability and the value of $\Delta\Delta G$ values the best amino acid residues were selected and then mutated using UCSF Chimera. Five model structures were obtained by mutating the following resi-dues 1YHO_D21V, 1YHO_K18V, 1YHO_P26K, 1YHO_Q35I and 1YHO_R91Y.

Table.2. Binding energy scores obtained by AutoDock for different mutants of native DHFR

Mutants generated by Chimera	Binding Energy (kcal/mol)	Interacting amino acids	Distance (in angstrom)
1YHO_D21V	-5.19	GLU30	2.19
		LEU22	2.24
		VAL8	2.6
1YHO_K18V	-7.59	GLU30	2.13
		THR136	2.30
		VAL8	2.17
1YHO_Q35I	-7.81	GLU30	2.20
		VAL8	2.11
		PHE31	2.18
1YHO_R91Y	-7.39	GLU30	1.88
		PHE31	2.18
		VAL8	2.08

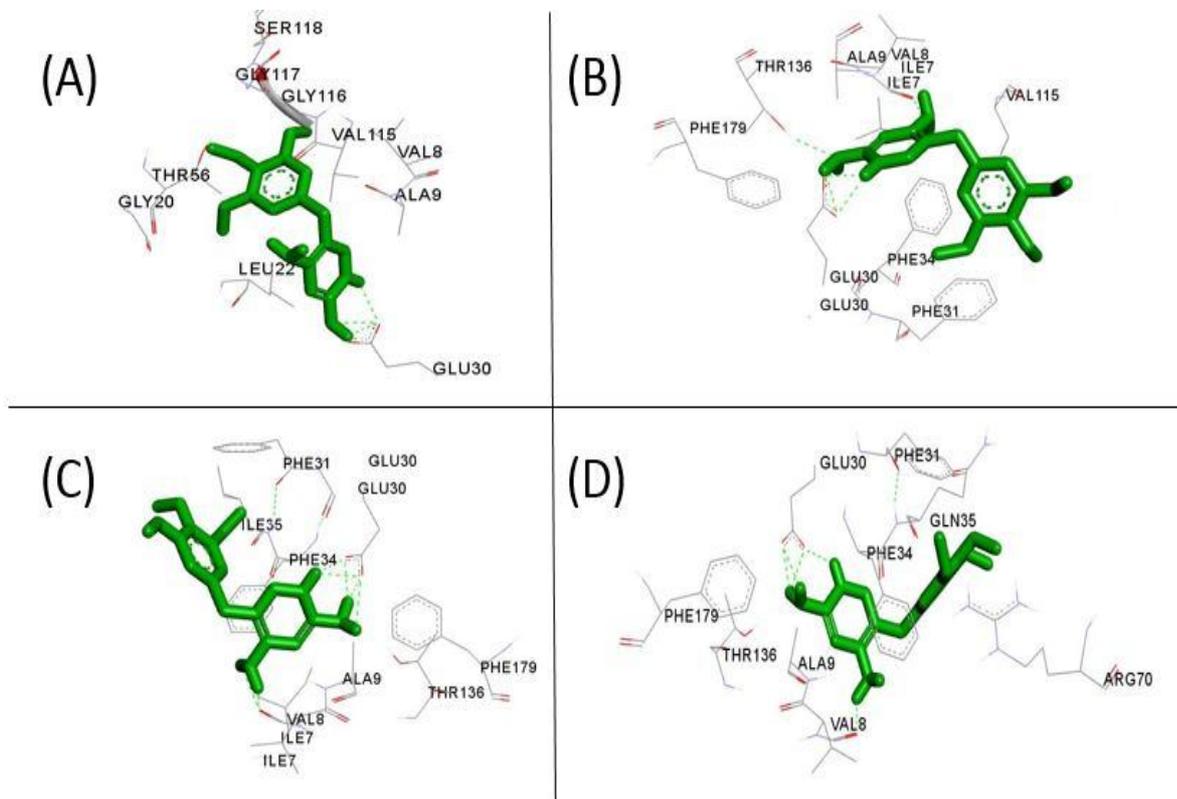


Figure 1. Mutant structures of native dihydrofolate reductase visualized by Discovery Studio. (A) 1YHO_D21V (B) 1YHO_K18V (C) 1YHO_Q35I (D) 1YHO_R91Y. TMP shown in Green color. Hydrogen bonds are indicated as Green dotted lines.

3.2. Molecular docking

The native and the mutant structures of DHFR were docked in our previous study and the binding energies were -5.31 and -1.57 kcal/mol respectively [9]. Mutant structures of DHFR which are generated by chimera were docked with TMP in the same binding pocket where native structure was docked. The binding energies were shown in Table.2.

The results of molecular docking shows that the binding energy of mutant DHFR (3S3V) is more than that of native DHFR (1YHO) and hence the entire focus shifted on molecular docking of the mutants generated for the native DHFR structure. From the Table2, the model structure 1YHO_Q35I is the most stable mutant with the most negative binding energy and will possibly bind best with the ligand (trimethoprim). Docking poses of 1YHO_D21V, 1YHO_K18V, 1YHO_P26K, 1YHO_Q35I and 1YHO_R91Y with TMP were visualized in discovery studio (Figure 1). TMP has shown has major interaction with GLU30, VAL8, PHE31 of 1YHO_Q35I with a binding energy of 7.81 kcal/mol. GLU30 plays important role in interaction of TMP with all the mutated structures of DHFR generated by chimera.

IV. CONCLUSIONS

In this study, we analyzed the change in cation-pi interactions the native and mutant structures of DHFR bound with trimethoprim. We mutated four structures from native DHFR structure for comparison of binding energy of native and mutant structures binding energy with trimethoprim. Docking was performed using these sites only for both the native and mutant structures and also the same site was used for the docking of the five modelled proteins with TMP. We observed high affinity binding of TMP with modelled structure 1YHO_Q35I with lowest binding energy. The high binding affinity could be due to the point mutation Q35I. Thus this computational study reveals a model structure of

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human DHFR protein with a novel binding site using PdbSum, when mu-tated with Isoleucine at 35th position of Glutamine, resulting in high binding affinity because of lower binding energy.

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