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Homology Modeling and Docking Studies Showed that Dihydrofolate Reductase from Pseudomonas Putida is a Possible Choice for Diagnosis of Serum Trimethoprim by Enzyme Inhibiton Assay

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Abstract

Trimethoprim is a chemotherapeutic drug mainly used in prophylaxis and treatment of bacterial infections. It belongs to dihydrofolate reducase inhibitors and has bacteriostatic properties. It may cause serious side effects if it is overdosed or used for long time. It may cause renal clearance mechanism impairment, thrombocytopenia, allergic reactions and a number of other clinical complications. An enzyme inhibition assay can be used to determine serum trimethoprim, which may provide advantage in terms of time and cost. This involves inhibition of dihydrofolate reductase by trimethoprim in serum. Dihydrofolate reductase (DHFR) is an enzyme that reduces dihydrofolic acid to tetrahydrofolic acid. In this study DHFR from *lactobacillus casei* (PDB id: 4DFR:A), *Bacillus anthracis* (PDB id:3JW3_A) and *Moritella profunda* (PDB id: 3IA4_A) are used as templates for building 3D models of DHFR from some other species. Programs used here are MODELLER, SWISS 3D MODEL and GENO3D. Based on overall stereochemical quality (PROCHECK, VARIFY3D, ANOLEA, PROSA) best models were selected, refined and characterized for binding site by CASTp program along with Catalytic Site Atlas (CSA) database. Best models were studied further for structure function relationship with ligand (trimethoprim) and its analogue (dihydrofolate reductase) by using docking approach (AutoDock and AutoDock VINA). The interaction energy between the trimethoprim and modeled enzyme indicated that homology models for DHFR of *Pseudomonas putida* can account for better regionspecificity of this enzyme towards trimethoprim. Findings from the current study could be utilized to de novo enzyme selection for diagnosis of serum trimethoprim.

Introduction

Dihydrofolate reductase is the target enzyme for a group of antifolate drugs like methotrexate and trimethoprim [1]. It inactivates dihydrofolate reductase, which functions in conversion of dihydrofolate to tetrahydrofolate. And folate is an essential factor for DNA synthesis and cell division. Due to inhibition of DNA synthesis and replication bacterial growth stops and thus trimethoprim can act as bacteriostatic agent [1,2]. But also trimethoprim may show some side effects. It may cause renal clearance mechanism impairment, thrombocytopenia, allergic reactions and a number of other clinical complications. It may also characterized by nausea, vomiting, swollen face, epigastric pain, headache, & weakness [4]. Diagnosis of serum trimethoprim can aid to determine the efficacy of drug and its effect in system in terms of dose and time. An enzyme inhibition assay can be used to determine serum substrate by testing inhibition of enzyme in serum [5, 6]. For understanding and analyzing protein function it is necessary to understand its 3D structure. Protein 3D structure can be determined by experimental methods such as X-ray crystallography or NMR analysis. It can also predict by computational analysis. By homology modeling a reliable model of protein can be found [7-9]. These models have also been proved as useful for drug design projects and allowed to take actions in compound optimization and chemical adjustment [10]. By docking study the interaction of secondary structure elements in proteins may be demonstrated. It is considered to use matching two separate molecules. It used to show correlations between experimental binding affinities and its mathematical score for various protein-ligand complexes [11].

In present study, by using different programs like MODELLER, GENO3D, and SWISS 3D MODEL was used to generate 3D model of dihydrofolate reductase from different organisms. Dihydrofolate reductase from lactobacillus casei (PDB id: 4DFR:A), Bacillus anthracis (PDB id:3JW3_A) and Moritella profunda (PDB id: 3IA4_A) is used as template for model built up. Validation of these models was done by programs like PROCHECK, VARIFY3D, ANOLEA, PROSA etc. Active site prediction and docking study were performed using CASTp program, Catalytic Site Atlas (CSA) database, AutoDock and AutoDock Vina to

analyze functional association of dihydrofolate reductase with trimethoprim.

Materials and methods

2.1 Protein sequence retrieval and 3D modeling

Protein sequence was retrieved from NCBI protein sequence database (accession no: ABZ01067.1, ZP_06637221.1, NP_752010.1, YP_001454852.1, YP_002152055.1, ZP_06192186.1, YP_001439347.1, YP_003537753.1, ZP_06124802.1 and YP_003363702.1). Best template was selected by using NCBI protein blast by using hits against Brookhaven Protein Data Bank (PDB) database [16] to find nearest crystal structure. Dihydrofolate reductase structure from lactobacillus casei (PDB id: 4DFR:A), Bacillus anthracis (PDB id: 3JW3_A) and Moritella profunda (PDB id: 3IA4_A) was selected as template for their maximum sequence identity and E value. ClustalW was used for building pairwise sequence alignment. For 3D modeling MODELLER [14], SWISS 3D MODEL [12, 13] and GENO3D [15] were used.

2.2 Validation of 3D models

By using different software programs (MODELLER, SWISS 3D MODEL, PROCHECK [19], VERIFY3D [18], and PROSA [21]) the validation of structure models were obtained. RamchandranPlot obtained from PROCHECK was used to check stereochemical property. Model constructed from SWISS-3D MODEL and MODELLER was finally chosen for subsequent analysis as they possessed good geometry and energy profile. PROSA was used for final model to check energy criteria and Verify-3D was used to check compatibility of 3D models with its sequences.

2.3 Active site characterization

By aligning with known template with known active site we determined the active site of model structures. Here Catalytic Site Atlas (CSA) database [28-30] and CASTp program [22] was used with combination of PyMOL [3, 24] for visualization and analysis of protein molecular structures. In CSA database catalytic residues and enzyme active sites in 3D structure are documented. In CSA database it consists of two type's annotated site: original annotated set comprising information directly extracted from primary literature and annotations deduced by PSI-BLAST and sequence alignment with original set [28-30]. After determining catalytic site residues we aligned model sequences with template sequences to find conserved residues and dissimilar catalytic residues. These data was used to set grid parameter for docking approach.

2.4 Retrival of ligand structure

Structure of trimethoprim was obtained from NCBI PubChem [23]. OpenBabelGUI and AutoDock tools were used to convert this chemical format to a suitable format for docking approach. PubChem is a database for small molecules and their biological properties. It provides opportunity of rapid data retrieval, structure selectivity analysis, target selectivity examination etc [23].

2.5 Docking ligand into enzyme 3D model

AutoDock tools and AutoDock Vina [34] was used for docking ligand into enzyme active sites. Previous file formats were reformatted and refined prior to docking approach, utilizing AutoDock tools. AutoDock Vina was used for docking of ligand (trimethoprim) into enzyme active site. AutoDock Vina is a program that facilitates molecular docking and virtual screening approach. It is an automated docking tool which offers greater speed and improved accuracy for binding mode predictions with automated estimation of grid maps and clusters [34].

Results and discussion

3.1 Homology modeling

Homology modeling estimates the 3D structure of a target protein sequence by using its alignment to one or more protein template of known structure [25]. For structure based protein molecule design and function investigation homology modeling is most suitable method [26]. The modeling process involves of target-template selection and alignment, model building and model evaluation. [25] As the number of known protein structures are increasing and protein model software's are improving, the accuracy of the models are increasing [25]. As dihydrofolate reductase from Lactobacillus casei was previously used in enzyme inhibition assay for methotrexate we analyzed it in terms of trimethoprim[27]. DHFR from some related organisms therefore modeled. Organisms eg. Paenibacillus polymyxa (YP_003870895.1), Cronobacter sakazakii (YP_001439347.1), Erwinia amylovora (YP_003537753.1) Providencia rettgeri (ZP_06124802.1) Citrobacter rodentium (YP_003363702.1). For these organisms it was found that DHFR from L. casei (PDB id: 3DFR:A) is a suitable template. And for DHFR from some common microorganisms like *Pseudomonas putida* (ABZ01067.1), Serratia odorifera (ZP_06637221.1), Escherichia coli CFT073 (NP_752010.1), Citrobacter koseri (YP_001454852.1) and Proteus mirabilis (YP_002152055.1) it was found that DHFR from Bacillus Anthracis (3JW3_A) and Moritella

Profunda (3IA4_A) are suitable templates. Five models for each sequences was constructed using

MODELLER, SWISS 3D MODEL, and GENO3D. Using RamchandranPlot from ProSA, Phi and Psi torsion angles were checked. For each sequence best model was selected for subsequent analysis. These models were further refined for docking purpose using AutoDock tools. Polar hydrogen was added to each structure.

3.2 Model evaluation

The quality of protein model verifies the informatics can be mined from it. So, evaluation of the accuracy of protein modes is essential for their interpretation [25]. For this purpose different programs were used e.g. Swiss 3D model, PROCHECK, VARIFY3D and PROSA. Stereochemical properties of the models were evaluated by ProCheck. A Ramchandran plot was found for every model (Table 01). This plot shows the quality of each model. For each sequence best model then selected. The Ramachandran plot showed that model found from MODELLER (ABZ01067.1, YP_003870895.1, YP_001439347.1 and YP_003537753.1) and from SWISS-3D MODEL (ZP_06124802.1, YP_003363702.1, ZP_06637221.1, NP_752010.1, YP_001454852.1, YP_002152055.1) have most residues in most favorable region and have overall good quality. From Ramchandran plot it was found that for model 6 (ABZ01067.1) 96.20% residues in most favorable region, 2.90% in allowed region, 0.60% in additional and 0.30% are in disallowed region (figure 5) as compared to template 3(3IA4_A) 97%, 2.70%, 0.3 % and 0.0%, respectively. It ensures that most residues are in consistent phi-psi distribution and are reliable for further analysis. Prosa energy plot showed that for each selected model the interaction energy for each residue with rest of the protein in negative and Verify-3D graph showed that for each selected model 3D-1D score is above zero (Table 2), thus side chain environments are acceptable.

3.3. Active site prediction and docking study

To analyze substrate binding and specificity docking study for all homology models was performed. By docking study interactions of substrate into active site can be visualized as protein substrate complex. Active site pockets of templates 4DFR:A, 3JW3_A and 3IA4_A were analyzed. All models ware aligned in order to find the corresponding regions of all structures. By sequence alignment and selecting matched point active site conservation analysis was performed.

It was found that ILE 5 (for template 3 ILE 6), MET 20 (MET 21), ASP27 (GLU 28), LEU28 (LEU 29), PHE31 (PHE 32), LEU54 (LEU55), ILE 94(ILE 96) was highly conserved among all template and models. In homology model 1, 6 and 7 active site residues MET21, GLU28 and LEU29 was different. Changes in conserved residues may change conformational change and binding pattern with substrate. Finally docking study for the protein 3D models was performed to find its relation in terms of ligand binding. Trimethoprim was successive docked onto active site of enzyme models. Table 3 shows output of docking experiments in terms of affinity (kcal/mol). Different model shows significant difference in dock scores. Among them model 5 showed highest dock scores -14.9 illustrated its tight binding with target.

Conclusion

Comparative structural modeling and docking simulations showed significant difference in affinity of dihydrofolate reductase towards trimethoprim. Various model evaluation methods indicated that modeled structures has considerably good geometry and acceptable profiles for all programs. DHFR from *Pseudomonas putida* showed significant dock scores than others. It suggests its possible application for analysis of serum trimethoprim by enzyme inhibition assay.

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Illustrations

Illustration 1

Fig. 1. Alignment for DHFR sequence of Pseudomonas putida with template 3IA4 A.

Illustration 2

Fig. 2. (a) 2D structure of trimethoprim (b) 3D structure of trimethoprim

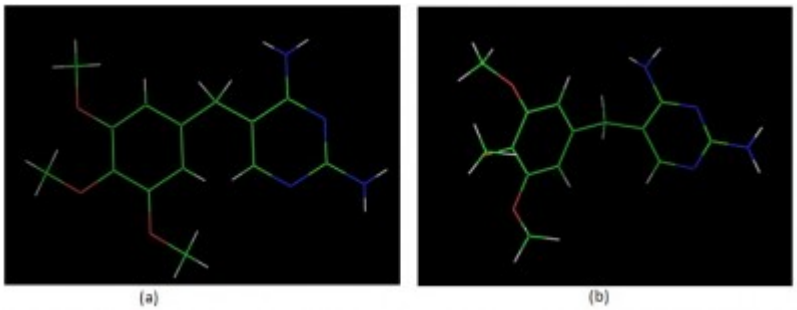


Illustration 3

Fig. 3. (a) 3D view of Model 1 (Paenibacillus polymyxa Modeller). (b) 3D view of Model 6(Pseudomonas putida Modeller).

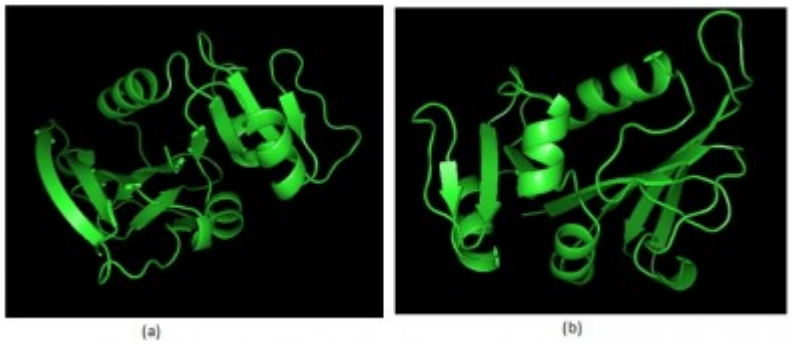


Illustration 4

Fig. 4. (a) Superimposition of DHFR of model 1 with template 4DFR: A (b) Superimposition of model 6 with template 3IA4_A

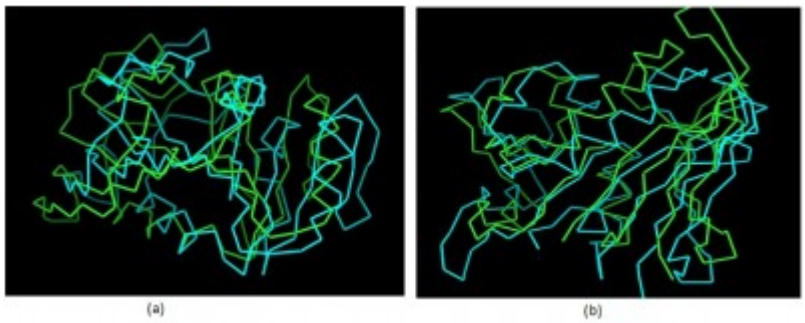


Illustration 5

Table 1 Ramchandran plot analysis of 3D models by PROCHECK.

Models	Most Favorable regions	Additional allowed regions	Generously allowed regions	Disallowed regions	G factor overall
Template 1	96.5%	3.10%	0.40%	0.0%	-0.02
Model 1	94.50%	4.20%	0.80%	0.50%	-0.1
Model 2	94.90%	4%	0.50%	0.60%	-0.18
Model 3	95.10%	3.70%	0.60%	0.60%	-0.12
Model 4	94.12%	4.68%	0.40%	0.80%	-0.16
Model 5	95.10%	3.30%	0.80%	0.80%	-0.08
Template 2	96.50%	3.10%	0.40%	0.0%	-0.04
Template 3	97.00%	2.70%	0.30%	0.0%	-0.05
Model 6	96.20%	2.90%	0.60%	0.30%	-0.08
Model 7	94.20%	4.40%	0.80%	0.60%	-0.18
Model 8	92.50%	6.50%	0.60%	0.40%	-0.16
Model 9	93.80%	5.40%	0.40%	0.40%	-0.11
Model 10	94.20%	5.20%	0.30%	0.30%	-0.16

Illustration 6

Fig. 5. Ramchandran Plot analysis of Model 6 (Pseudomonas putida Modeller).

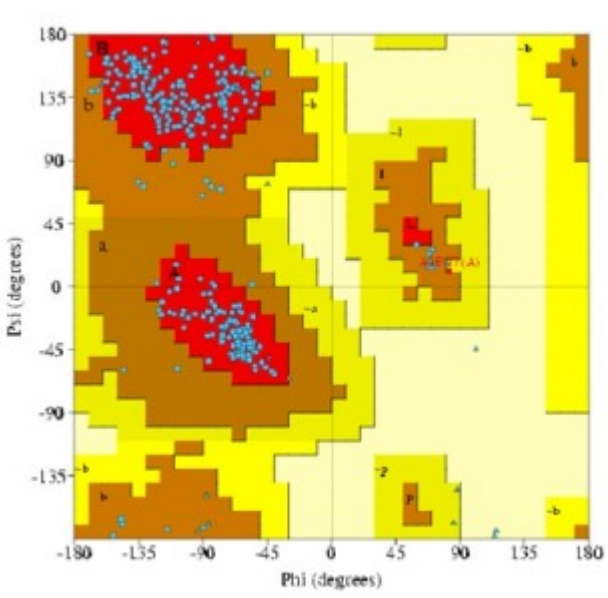


Illustration 7

Table 2 3D–1D evaluation scores for models

Models	3D–1Dscore
Template 1	0.80
Model 1	0.76
Model 2	0.73
Model 3	0.72
Model 4	0.75
Model 5	0.78
Template 2	0.82
Template 3	0.85
Model 6	0.78
Model 7	0.71
Model 8	0.72
Model 9	0.76
Model 10	0.75

Illustration 8

Fig. 6. (a) PROSA energy plot analysis of Model 6 (b) The 3D profiles found from Verify 3D–1D for model 6.

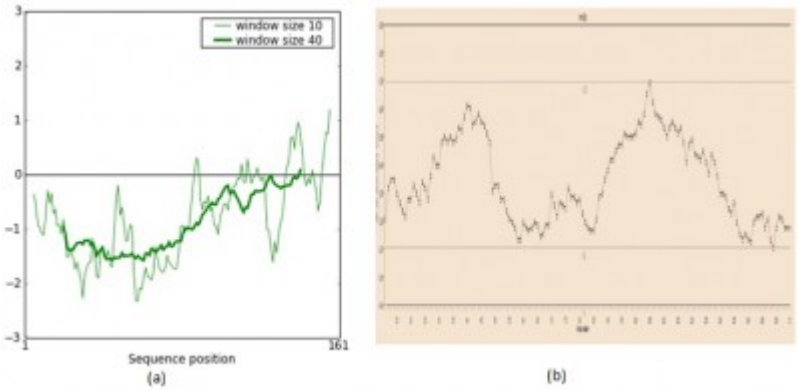


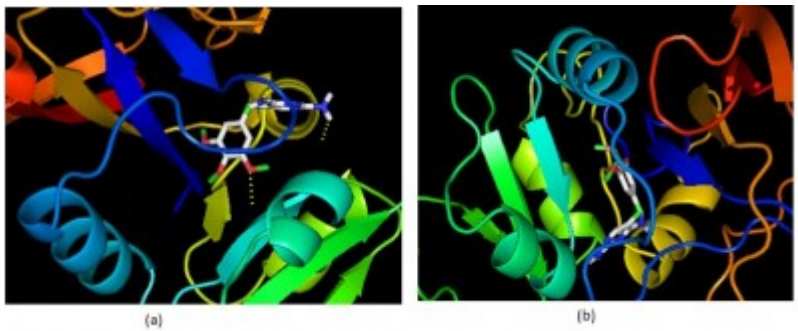
Illustration 9

Table 3 Docking score for 3D models with AutoDock Vina

Models	Accession no	Organism	Dock Score (kcal/mol)
Template 1	4DFR:A	*Lactobacillus casei*	-9.50
Model 1	YP_003870895.1	*Paenibacillus polymyxa*	-13.8
Model 2	YP_001439347.1	*Cronobacter sakazakii*	-9.60
Model 3	YP_003537753.1	*Erwinia amylovora*	-12.3
Model 4	ZP_06124802.1	*Providencia rettgeri*	-8.70
Model 5	YP_003363702.1	*Citrobacter rodentium*	-9.40
Template 2	3JW3_A	*Bacillus Anthracis*	-12.5
Template 3	3IA4_A	*Moritella Profunda*	-11.75
Model 6	ABZ01067.1	*Pseudomonas putida*	-14.7
Model 7	ZP_06637221.1	*Serratia odorifera*	-12.3
Model 2	NP_752010.1	*Escherichia coli* CFT073	-8.70
Model 3	YP_001454852.1	*Citrobacter koseri*	-8.60
Model 10	YP_002152055.1	*Proteus mirabilis*	-6.60

Illustration 10

Fig. 7. (a) Docking of model 1 with trimethoprim (b) Docking of model 6 with trimethoprim



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