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INSILICO STUDIES OF NATURAL COMPOUNDS AS PERIPLASMIC NITRATE REDUCTASE INHIBITORS

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ABSTRACT

The present study explains computational methods to design 3D structure of "Periplasmic nitrate reductase" of Actinobacillus pleuropneumoniae serotype 3 (strain JL03) enzyme using the sequence available from uniprot (uniProtKB – B0BR28). Modelling study was performed to generate a 3D model for Periplasmic nitrate reductase protein. The model was developed by using Modeler9.18 software. The developed model was further docked with quercetin, citric acid, cuminaldehyde, eugenol, luteolin and riboflavin compounds by using AUTODOCK4.2 software to identify the functional effect of protein. The developed model showed 91.3% of the amino acids in most favored region. All the compounds exhibited good binding energy and interactions. These studies provide understanding and interpreting the data produced by these methods. It explains to understand molecular interactions at the active site region.

KEY WORDS

Homology modeling, Periplasmic nitrate reductase, Natural compounds, Docking.

INTRODUCTION:

In prokaryotes Periplasmic nitrate reductase catalyzes the transformation of nitrate to nitrite.[1] periplasmic nitrate reductases found in denitrifying bacteria like Bacillus, *Pseudomonas*, thiobacillus etc., the role of periplasmic nitrate reductase is not being clear in denitrification.[2] Denitrification occurs under O_2 limiting conditions. Denitrification plays a significant role in completing the N_2 cycle by converting NO_3^- to N_2 gas. The periplasmic nitrate reductase consists NapA and NapB subunits. Membrane-bound nitrate reductase and periplasmic nitrate reductase are present in *Plasmodium denitrificans.*[3]

In the present study, an effort was made to generate the 3D structure of the Periplasmic nitrate reductase (Uniprot accession number: B0BR28) from *Actinobacillus pleuropneumoniae serotype 3 (strain JL03).* Modeller9.18 was used for the homology modelling. The model was validated by using PROCHECK. Present study could provide useful information to get the functional characterization of these enzymes. Molecular docking studies were performed by using Autodock4.2 with known inhibitors like Quercetin, Citric acid, Cuminaldehyde, Eugenol, Luteolin and Riboflavin.

METHODOLOGY:

Homology modelling

The amino acid sequence of Periplasmic nitrate reductase was retrieved from Uniprot.[4] A three dimensional model was generated for "Periplasmic nitrate reductase". A sequence similarity search was

performed to identify the structural similarity of the query sequence by using Protein BLAST[5] tool by selecting database against Protein Data Bank (PDB) for identifying template for homology model building.[6] The template was identified on the basis of smaller the E-value, >30% identity, maximum score. 2NYA protein was selected as a template for modeled protein. Comparative sequence alignment studies were performed with query and template structure using Clusta lX tool and online Clustal W tools.[7]

MODELLER9.18 software was used to develop the model. It is an automated approach to comparative modeling by satisfaction of spatial restrains.[8] To align the query and template sequences manually the input file of alignment.ali was used in MODELLER 9.18. After completion of alignment twenty models were generated and all the generated models were thermodynamically minimized using molecular dynamics and simulation approach. By implementing MODELLER9.18 auto-model class, calculated 3D models of the target automatically. The best model which is having smallest value was selected on the basis of Lowest Objective Function. It is also known as normalized Discrete Optimized Molecule Energy (DOPE) score. Generate model was then checked in detail for protein structure stereochemistry including Ramachandran plot and Psi/Phi angles using PROCHECK.[9]

Molecular docking studies

All the molecules were collected from scientific literature and sketched in SYBYL6.7[10] and energy minimized by adding Gasteiger Huckel charges. The molecules were then saved in .mol2 format for molecular docking purpose.

Molecular docking studies were performed to explain the binding mode of proteins and ligands. All the existing compounds were docked by using Autodock 4.2 software[11]. All the molecules were docked individually in Autodock4.2. The modelled three-dimensional structure of Periplasmic nitrate reductase protein was imported to Autodock 4.2 and structurally optimized by adding hydrogens to protein allocated with kollaman charges.[12] After adding the hydrogens the model was saved in PDBQT format, later ligands were prepared by optimizing the torsion angles and saved them in PDBQT format. A grid was generated around to identify XYZ coordinates (X=3.065, Y=3.382 and Z= 54.957), around binding site of Periplasmic nitrate reductase protein. Lamarckian genetic algorithm (LGA) was selected for freezing, docking and default parameters used in autodock4.2.

RESULTS AND DISCUSSION:

After sequence alignment and homology modeling of Periplasmic nitrate reductase shows highly conserved regions in amino acid sequences. The most homologous template for building a homology model for Periplasmic nitrate reductase was identified through protein blast algorithm. Based upon the homology search, Crystallographic structure of "*Chain A, Crystal Structure of The Periplasmic Nitrate Reductase (Nap) From Escherichia Coli*" (PDB entry: 2NYA) was selected as a template. Twenty models were generated using Modeler 9.18 program. The alignment file was tweaked manually to excellent fit in the sequences. After the generated models for all the primary sequences, the model with least object function was selected for further protein stereochemistry evaluation (phi and psi angles) with procheck software. Figure1 and 2 shows super pose of model and template structures with backbone trace and the cartoon of homology derived protein of Periplasmic nitrate reductase.

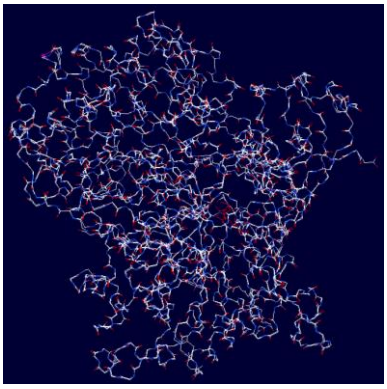

Figure 1: Super pose of model and template structures with backbone trace. The models were superimposed by using swiss pdb viewer (spdbv).

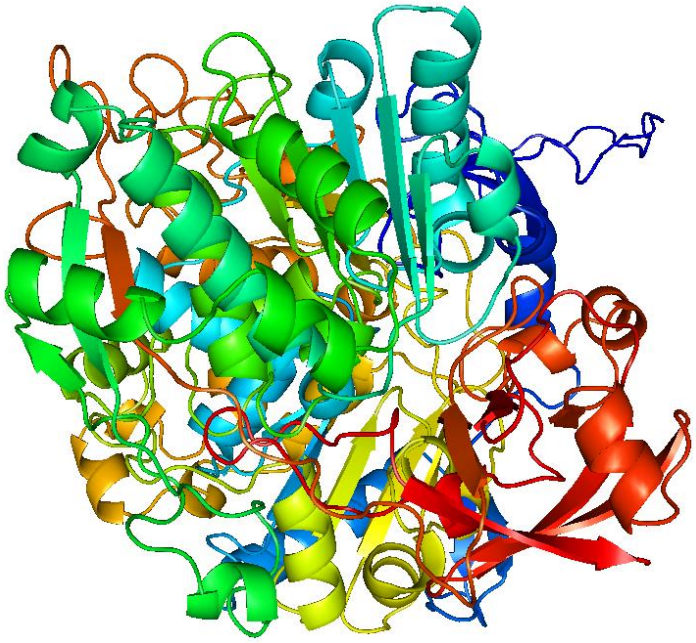

Figure 2: The cartoon of homology derived protein of B0BR28 modelled protein.

The three (Ø, Ψ and ω) backbone torsion angles are important determinants of a protein fold. PROCHECK software generates a number of scatter plots, these are known as Ramachandran plots. These plots show complete residue by residue data and the assessment of the generally excellence of the producing structure as compared to well refined structures of the same resolution. The Ramachandran plot is the main indicator to check the intrinsic quality of the protein structure. The Ramachandran plot of the template (PDB ID: 2NYA) have 1172 amino acid residues (86.3 %) in most favorable regions, 168 amino acid residues (12.4 %) falling into additionally allowed regions, 11 amino acid residues falling in to generously allowed region (0.8%) and seven amino acid residues in disallowed region (0.5%). whereas for the modeled protein have, 650 amino acid residues (91.3 %) in the most favorable region, 56 amino acid residues in additionally allowed region (7.9 %) and six amino acid residues present in generously allowed region (0.8%). There is no amino acid residue present in disallowed region. The Ramachandran plot is shown in figure 4. These results clearly indicate that the generated protein model is more conformationally superior to the template structure. The modeled structure was superimposed with the template 2NYA by using SPDBV, it was observed that RMSD value of 0.49 Å.

Molecular docking of natural compounds into the binding site of a receptor and estimating the binding affinity of the ligand is a most important part of the structure-based drug design process. The molecular docking results indicates that all the studied natural derivatives occupy an almost similar space in the binding site. Quercetin showed best possible binding mode against modelled Periplasmic nitrate reductase protein is illustrated in Figure 4. During the molecular docking procedure, the program selects only best fit active site pocket of the protein with respect to the ligands in order to dock them. AutoDock 4.2, provides information on the binding orientation of ligands at the active site region. The docking program place both ligand and protein in different orientations, conformational positions and the lowest energy confirmations which are energetically favorable are evaluated and analyzed for interactions. Free energies of binding (ΔGb) and dissociation constants (Ki) as calculated by AutoDock are summarized.

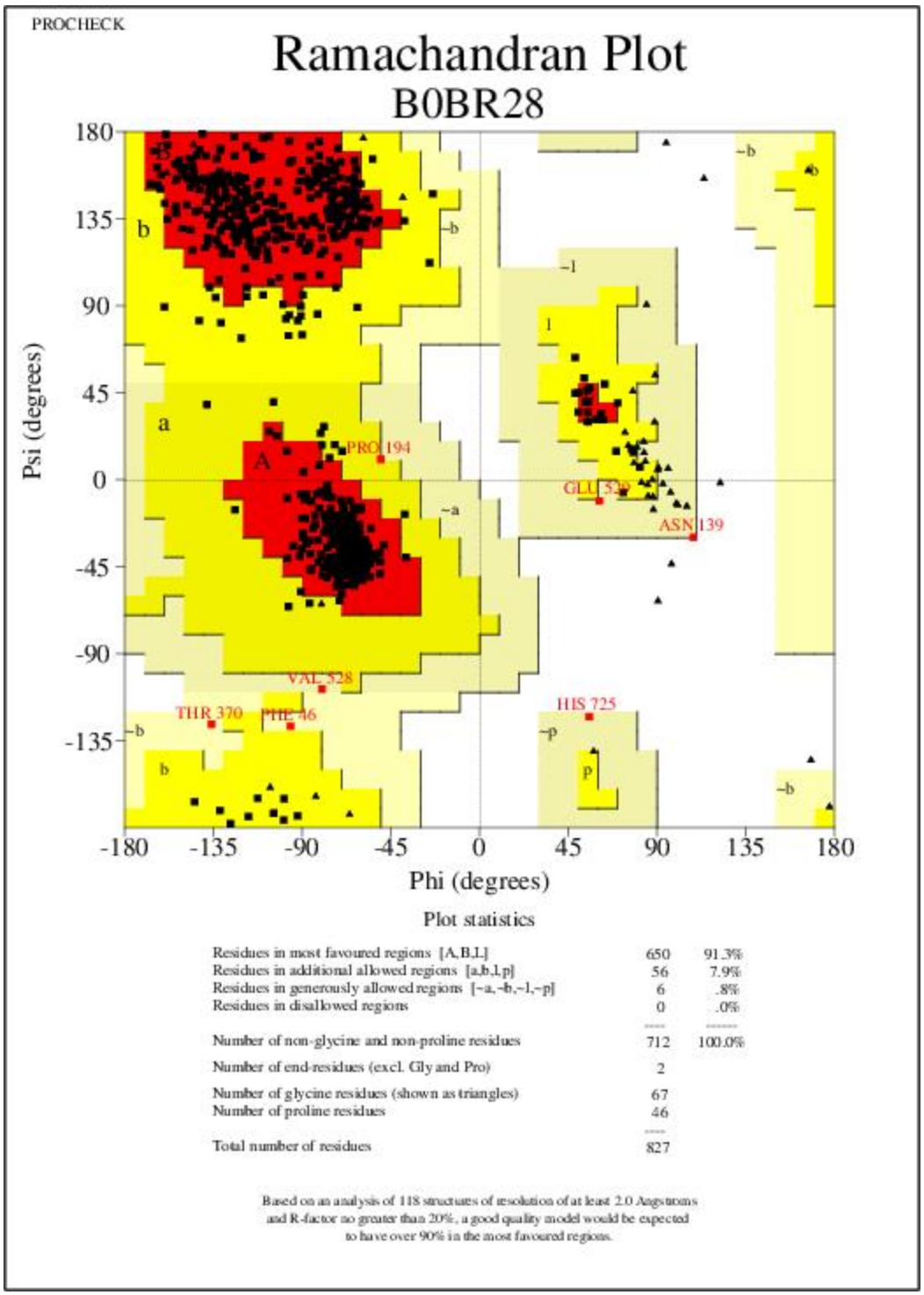

Figure 4: Ramachandran plot of the modelled B0BR28 protein

The binding energy and interacting amino acid residues are given in table 1. For all the molecules binding affinity was characterized by binding energy (ΔG) value. Ligand quercetin showed highest binding energy of -6.84 kcal/mol with interacting Met729 and Trp724. Ligands luteolin and riboflavin showed three interactions. Luteolin interacts with Arg173, Met279, His725 with binding energy of -6.59 kcal/mol. Riboflavin interacts with Ans173(2), Thr480, Asn481 with a docking score of -4.62 kcal/mol. All the docking poses of the molecules were shown in Figure 2.

Name of the ligand	Interacting aminoacids	Binding Energy ΔG (Kcal/Mol)	Dissociation constant (kI) (μM)
Quercetin	Tpr724, Met729	-6.84	9.72
Citric acid	Glu148(2), Met729	-4.86	273.16
Cuminaldehyde	Gln375, Arg719	-5.37	116.64
Eugenol	Gln375(2), Lys818	-5.50	93.64
Luteolin	Arg173, Met279, His725	-6.59	14.86
Riboflavin	Ans173(2), Thr480, Asn481	-4.62	89.18

Table 1: Binding energy and interacting residues of natural compounds with modelled B0BR28 protein

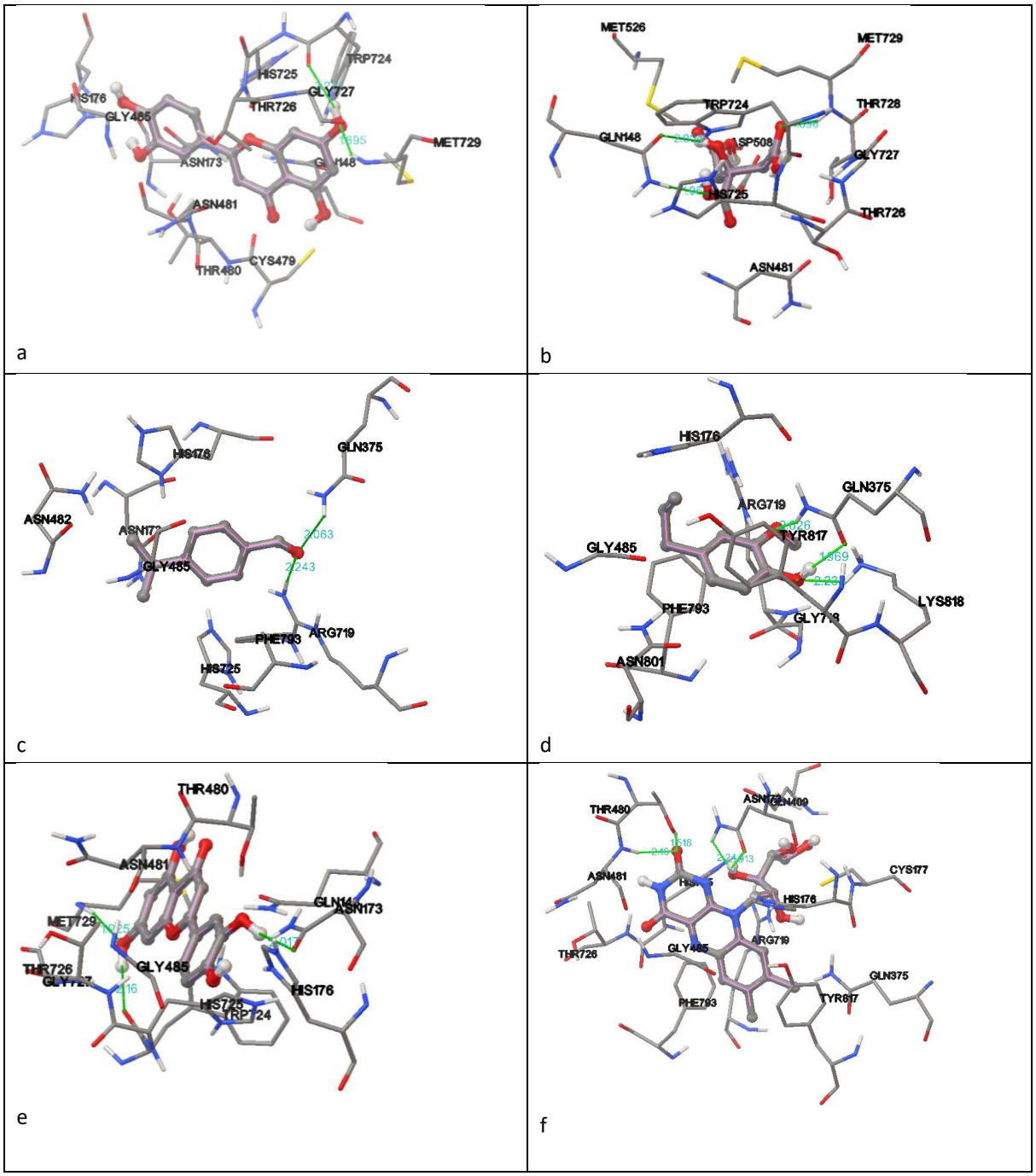

Figure 2: Docking interactions of B0BR28 protein with (a) quercetin (b) citric acid (c) cuminaldehyde (d) eugenol (e) luteolin (f) riboflavin.

CONCLUSION:

In this work, homology modeling and molecular docking studies were performed to explore structural features and binding mechanism of existing quercetin, citric acid, cuminaldehyde, eugenol, luteolin, riboflavin. Ligands as Periplasmic nitrate reductase inhibitors, and to construct a model for designing new Periplasmic nitrate reductase protein. Homology derived model statistics are similar to template i.e., crystal structure. Docking the modelled protein with these ligands provided insight into the binding and interaction with the enzyme. Further, the structure-based drug discovery process along with protein information of drug targets may improve our understanding towards in-sight of mechanism of protein-ligand interactions and their binding patterns.

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