ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Research Article

HOMOLOGY MODELING OF GLUTAMINASE FREE L-ASPARAGINASE FROM *FUSARIUM SOLANI* CLR-36

Amani Esmaeil As-Suhbani*, Hemlata Bhosale

DST-Fist Sponsored School of Life Sciences, Swami Ramanand Teerth Marathwada University, Nanded, Maharashtra, India

**Corresponding author: amaniesmaeil.7@gmail.com*

ABSTRACT

This study was aimed to predict the 3D model of L-asparaginase from *F. solani* CLR-36 along with the prediction of the protein active site and evaluation of the L-asparagine-ligand binding with the protein by protein docking studies. MODELLER9.21 was used to generate the 3D model of L-asparaginase (GenBank: MN166289.1) from *F. solani* CLR-36. "Crystal structure of the Asn-bound guinea pig L-asparaginase 1 catalytic domain active site mutant T19A" (PDB id: 5DNC), was used as template to build the model. The built model was validated by PROCHECK software. To predict the active site, BLAST (Basic Local Alignment Search Tool of NCBI) for similarity with Conserved Domain Database (CDD) of NCBI was used, and molecular docking study was carried out using AutoDock Tools. 3D Protein Structure was predicted and the Ramachandran plot of the protein model generated showed 86.1% residues in most favored region indicating the reliability of the model. The protein active sites were evaluated and Docking of L-asparaginase was performed with L-asparagine. The docking result showed that the interactions were occurring at the active site with good binding energy (-6.85kcal/mol) indicating that ligand L-asparagine will show good activity with the target protein-L-asparaginase. This study provides base line information on structural and functional features of L-asparaginase from *F. solani* CLR-36 with its L-asparagine-ligand which could be useful in the designing of alternative useful drug in the chemotherapy of ALL.

Keywords: L-asparaginase; *Fusarium sIolani* CLR-36; Homology modeling; Molecular Docking

1. INTRODUCTION

L-asparaginase (EC 3.5.1.1) is an enzyme belongs to the hydrolase class that catalyzes the conversion of L-asparagine to aspartate and ammonia [1]. L-asparaginase anticancer activity is associated with the exhaustion of the L-asparagine in the circulating pool through the catalytic activity of L-asparaginase [2, 3]. In normal cells the deficiency of the endogenous L-asparagine could be compensated by endogenous synthesis from aspartic and glutamine acid via the L-asparagine synthase. This enzyme is absent in neoplasmatic cells, especially in lymphoblasts and for rapid proliferation, tumor cells require L-asparagine in high amount. The concequences of that is the depletion of L-asparagine from the plasma which in turn leads to inhibition of DNA, RNA and protein synthesis followed by in blast cell apoptosis [4-6]. Microbial sources are most common for production of L-asparaginase in large scale, and that is related to the cost effective production [7]. L-asparaginase is widely used in the chemotherapy of acute lymphoblastic leukaemia (ALL) and Lymphosarcoma cancer and it is commercially obtained from *E. coli* and *Erwinia chrysanthemi* [8, 9].

The use of commercial L-asparaginase has many draw-backs, its use lead to development of resistance [10] along many side effects like liver dysfunction, pancreatitis, leucopenia, neurological seizures, coagulation disorders which causing intracranial thrombosis and/or hemorrhage, and that is mainly related to its high glutaminase activity as well as low substrate specificity [11]. L-glutaminase activity of L-asparaginase leads to depletion of L-glutamine in blood plasma through deamination of L-glutamine to L-glutamate that is responsible of the toxic effects in the normal cells [12]. Therefore, there is necessity for searching of novel L-asparaginases from other microorganisms which could overcome the challenges mentioned above and to fulfil the drug industry demand [1, 11].

In an earlier study, we have reported the production and optimization of glutaminase free L-asparaginase from *Fusarium solani* [13, 14].The present study is aimed

towards the *in silico* protein structure modeling of L-asparaginase from *F. solani*, and active site evaluation and L-asparagine-ligand binding with the protein by employing protein docking studies.

2. MATERIAL AND METHODS

In the present study, MODELLER9.21 was used to predict and generate the 3D model of L-asparaginase protein from *Fusarium solani* CLR-36. "Crystal structure of the Asn-bound guinea pig L-asparaginase 1 catalytic domain active site mutant T19A" (PDB id: 5DNC), was used as template to build the model. The built model was validated by PROCHECK software. To predict the active site, BLAST (Basic Local Alignment Search Tool of NCBI) for similarity with Conserved Domain Database (CDD) of NCBI was used, and molecular docking study was carried out using AutoDock Tools.

2.1. L-asparaginase gene sequencing and characterization

F. solani was identified based on 28s rRNA as *F. solani* CLR36 and the sequence deposited in the Genbank under the accession number: MG719989.1. L-asparaginase gene from *F. solani* CLR-36 was sequenced and deposited to Genbank under the accession number: MN166289.1. The translated amino acid sequence was used as a source sequence for further study.

2.2. Protein Modelling

For protein modelling, MODELLER 9.21 was used. Modeller is software that does protein modelling on the basis of homology. MODELLER is used for homology or comparative modelling of protein three-dimensional structures [15]. The sequence alignment was used and was modelled with known related structures and MODELLER automatically calculates a model containing all non-hydrogen atoms. MODELLER implements comparative protein structure modelling by satisfaction of spatial restraints [16, 17], and can perform many additional tasks, including de novo modelling of loops in protein structures, optimization of various models of protein structure with respect to a flexibly defined objective function, multiple alignment of protein sequences and/or structures, clustering, searching of sequence databases, comparison of protein structures, etc.

In homology modelling, sequence alignment of the protein with the database was performed so as to find similar proteins for our sequence. So selection of template was done using bioinformatics tools on the basis of identity and best coverage. For homology modelling, identity percentage should be minimum 25%. Structure alignment of template was done with the query so as to find out the final alignment and the conserved regions. Analyzing the structural alignment was performed to find template similarity, with very less gaps, and good conserved regions. Finally, models were generated using MODELLER.

2.3. Model Validation

For validation of structure Ramachandran Plot (RC plot) was used. RC plot was generated using PDBsum (which used PROCHECK for the plot generation).

2.4. Active Site Prediction

Prediction of active site was done using BLAST (Basic Local Alignment Search Tool of NCBI) for similarity with Conserved Domain Database (CDD) of NCBI as it is based on literature available with respect to the conserved domains. CDD is an NCBI database. It is a resource of protein annotation which includes a compiled data of well-annotated multiple sequence alignment (MSA) models for ancient domains and full-length proteins. It uses curated data domains of NCBI.

For finding the active site, CDD gave the family of protein on the basis of conserved domains and we found *cl00216* as the super family to which our protein, L-asparaginase belongs.

2.5. Protein Docking

In order to validate the of the predicted active site of *protein* model and check its possible mode of interaction with its normal substrate L-asparagine (L-asparagine obtained from PUBCHEM database, PubChem CID:6267), docking of the homology model of L-asparaginase protein (receptor) was performed with L-asparagine (ligand) using AutoDock Tools [18]. The homology model of protein was loaded into the AutoDock Tools as a receptor and made ready for docking. Using the edit option in AutoDock Tools, hydrogens were added after loading the protein. The L-asparagine was then docked with protein; a grid for dock search was built for the molecule in order to find the most probable binding site of the protein and to measure its interaction parameters with the ligand L-asparagine. The docking process was performed using the AutoDock Tools default parameters.

3. RESULTS AND DISCUSSION

3.1. Modelling Method

Performing the protein sequence alignment with the database, identity percentage with the template selected, "Crystal structure of the Asn-bound guinea pig L-asparaginase 1 catalytic domain active site mutant T19A" (PDB id: 5DNC), was 39% 9 (Fig. 1). For homology modeling, identity percentage should be minimum 25%. "The selected template was downloaded from rcsb, a protein structure database (http://www.rcsb.org/pdb). https://www.rcsb.org/structure/5DNC)".

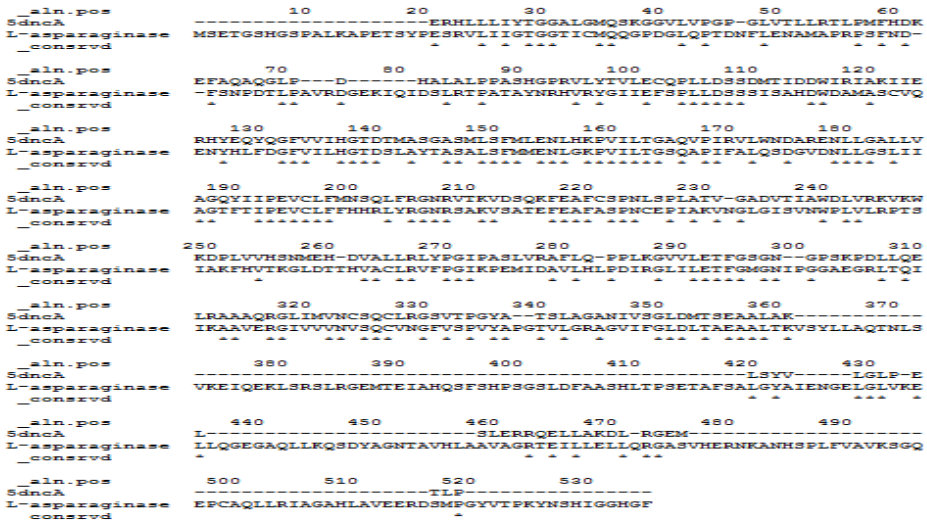


Fig. 1: Structural Alignment of L-asparaginase with the 5DNC (chain A)

Analyzing of final structural alignment and the conserved regions of the protein and the template showed that there was a high query and template similarity, with very less gaps, and good conserved regions (Fig. 2). The selection of the best model is based on the molpdf-Molecular potential density function in which the lowest value is indication a good model. Fig.2 shows that model having molpdf value 139.533 was the lowest among the other produced models, so that model was selected and considered as a good model. The 3D Protein Structure Image was viewed in Discovery Studio (Fig. 3).

```
>> Summary of successfully produced loop models:
Filename                                molpdf
----------------------------------------------
L-asparaginase.BL00010001.pdb      1197.39270
L-asparaginase.BL00020001.pdb       139.53258
L-asparaginase.BL00030001.pdb      5262.42822
L-asparaginase.BL00040001.pdb       614.84253
L-asparaginase.BL00050001.pdb       422.53769
L-asparaginase.BL00060001.pdb       951.26880
L-asparaginase.BL00070001.pdb      3336.30542
L-asparaginase.BL00080001.pdb      2648.38574
L-asparaginase.BL00090001.pdb       562.60120
L-asparaginase.BL00100001.pdb       152.51588
```

Fig. 2: List of successfully produced models in MODELLER

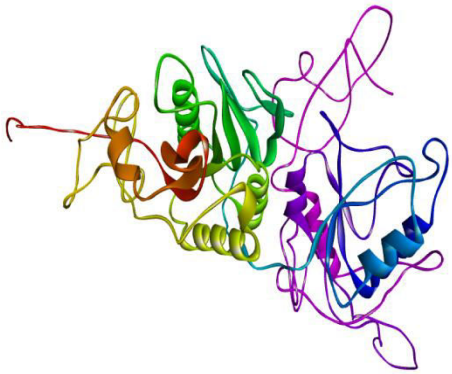


Fig. 3: 3D Protein Structure Image generated viewed in Discovery Studio

3.2. Model Validation:

Fig. 4 represents Ramachandran Plot (RC plot) for the selected final model. The Ramachandran plot shows the phi-psi torsion angles for all residues in the structure. Glycine residues are separately identified by triangles as these are not restricted to the regions of the plot appropriate to the other side chain types. The coloring/ shading on the plot represent the different regions, the darkest areas (here shown in *red*) correspond to the "core" regions representing the most favorable

combinations of phi-psi values. The percent-tage of residues in the "core" region is one of the better guides to stereo chemical quality. Also, residues in the disallowed region should ideally be less than or equal to 0.2%. Considering the protein model generated and analyzed from Ramachandran plot (PDBsum-PROCHECK). The Ramachandran plot of L-asparaginase shows 389 amino acid residues (86.1%) in most favorable region with 50 amino acid residues (11.1%) in to additional allowed regions and with 13 amino acids (2.9%) in to generously allowed regions and with 0 amino acids (0%) in disallowed region. These results evidently indicate is reliability of the generated model and confirm that it is conformationally better.

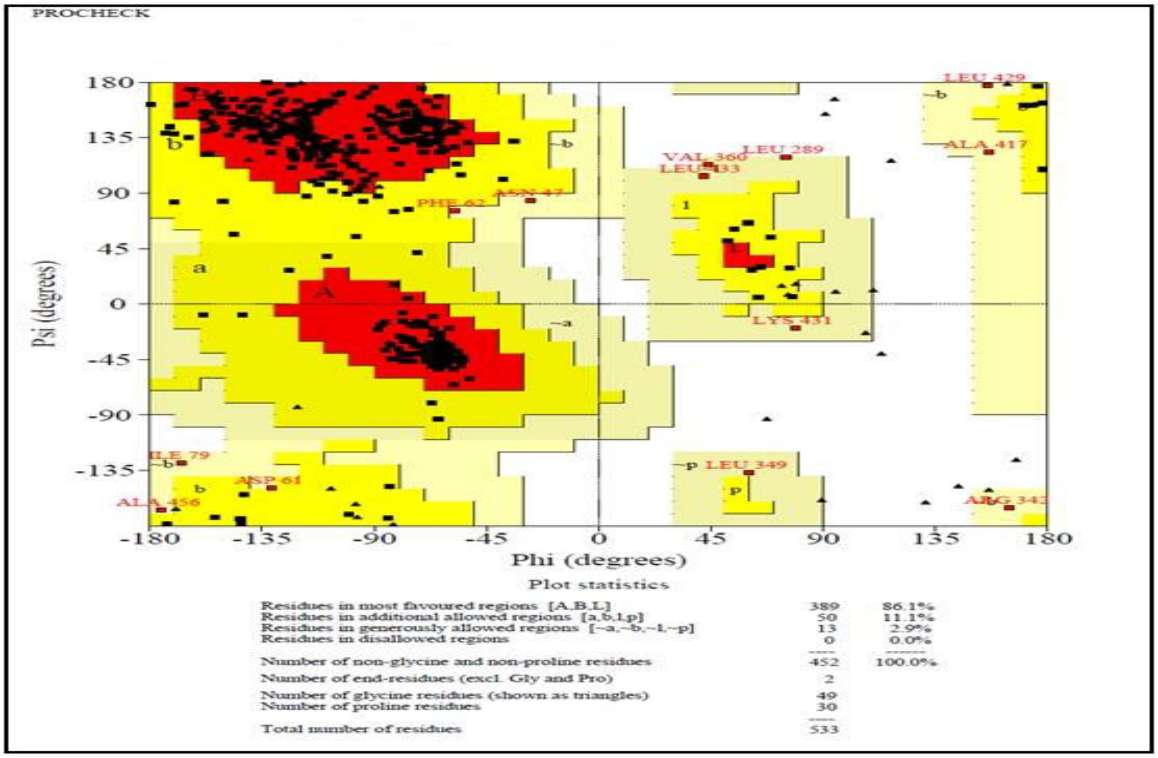


Fig. 4: Ramachandran Plot for the *F. solani* CLR-36 model, using PDBsum (PROCHECK)

3.3. Active Site Prediction Report

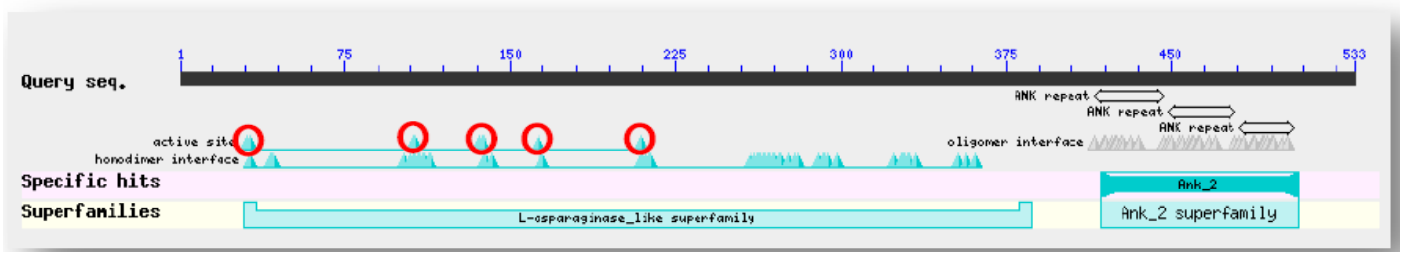
Fig.5 (a) shows active sites on conserved domain of L-asparaginase, predicted residues of active site were mapped to the *F. solani* CLR-36 L-asparaginase. The blue triangle is representation for the residues that comprise conserved features, active sites (marked in red circles) [19, 20].

Fig.5 (b) shows zoomed to residue level view for actives sites on conserved domain of L-asparaginase, predicted residues of active site were mapped to the *F. solani* CLR-36 L-asparaginase The blue triangle is representation for the residues that comprise conserved features, active site. We can say that the residues present in active site are- GLY 31, THR 32, SER 106, SER 107, GLY 136, THR 137, ASP 138, SER 163 & LYS 209 [1, 2], which used in molecular docking.

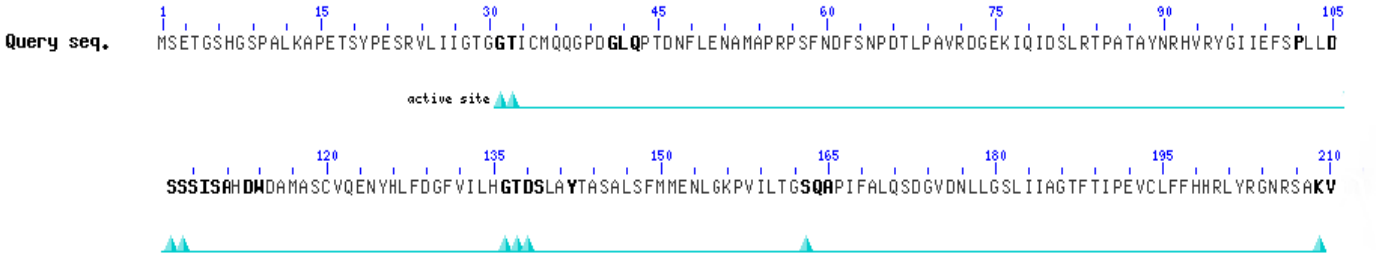
3.4. Molecular Docking

The docking result is shown in Table I, which shows that 7 hydrogen bonds are formed and the interactions are occurring at ASP138, THR137, SER163, ASP105, THR 3 2 & SER106. 2D interaction and 3 Dinteraction images for L-asparaginase with L-asparagine were generated using Discovery Studio (Fig. 6).

The interacting residues or interactions are found to be at the predicted active site residues (highlighted in yellow), with high binding energy -6.85 kcal/mol. Statistical Docking result for L-asparaginase with L-asparagine was performed using Discovery Studio (Table 1). The lower binding energy (lower the binding energy better is the stability of interaction), and the more number of hydrogen bonds and interactions occurring in the active site / pocket; indicating that the ligand- L-asparagine will show good activity with the target protein- L-asparaginase.



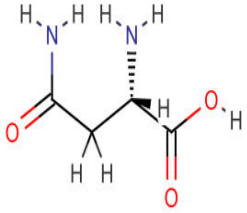
(a)

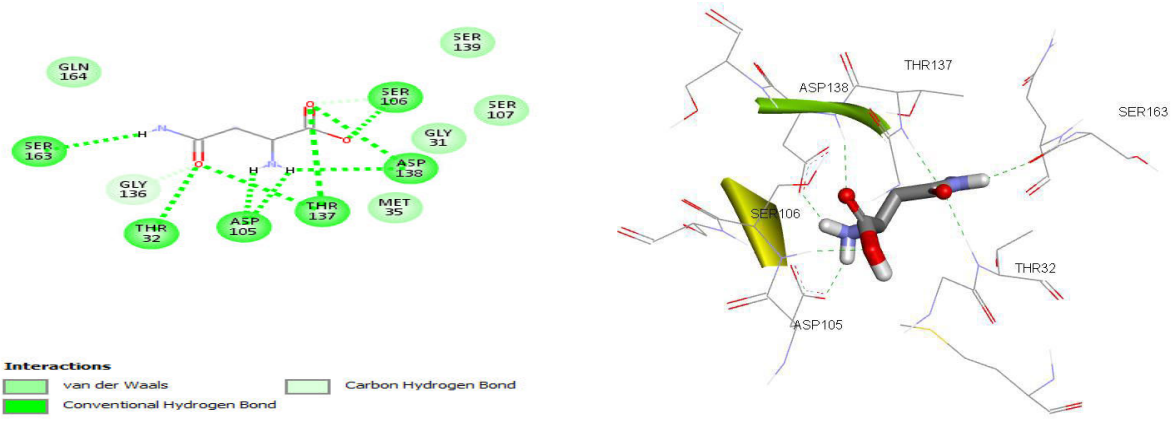


(b)

Fig. 5: (a) Active sites on conserved domain of L-asparaginase, (b) zoomed to residue level view for actives sites on conserved domain of L-asparaginase

Table.1: Statistical Docking result for L-asparaginase with L-asparagine

Ligand Name	Ligand Image	Interaction (s) Protein residue: atom→ ligand atom	Binding Energy ΔG (Kcal/mol)
L-asparagine		ASP138 : OD2→ H THR137 : HN→ O SER163 : O→ H ASP105 : OD2→ H ASP138 : HN→ O THR32 : HN→ O SER106 : HN→ O	-6.85



(a) (b)

Fig. 6: 2D interaction image (a) and 3Dinteraction image (b) for L-asparaginase with L-asparagine, using Discovery Studio

3.5. Structural validation after docking

Fig.7 represents Ramachandran Plot (RC plot) RC plot for the L-asparaginase after docking with L-asparagine substrate. The Ramachandran plot of L-asparaginase shows 389 amino acid residues (86.1%) in most favorable region with 50 amino acid residues (11.1%) in to additional allowed regions and with 13 amino acids (2.9%) in to generously allowed regions and with 0 amino acids (0%) in disallowed region. The total number of residues is 534 amino acids.

On comparing Ramachandran plot before and after docking of the ligand, it is observed that there is not much change in the distribution of amino acids and the energy conformation values did not differ much. These findings indicate that the geometry was not distorted during the docking of the enzyme with the L-asparagine substrate.

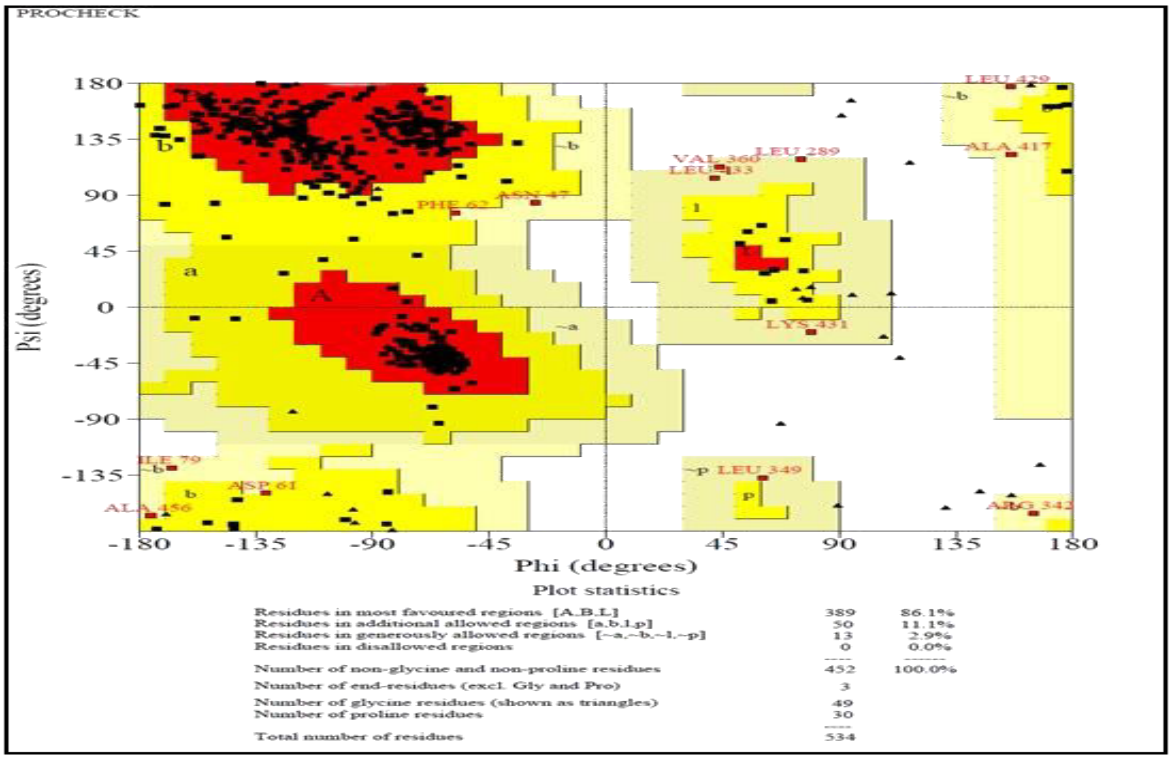


Fig.7: Ramachandran Plot for the *F. solani* CLR-36 L-asparaginase after docking with L-asparagine substrate, using PDBsum (PROCHECK)

4. CONCLUSION

Model prediction and molecular docking study were used to get the details of interaction between *F. solani* CLR-36 L-asparaginase and its natural substrate L-asparagine. The validation of the results by PROCHECK indicated that the generated model is reliable and is analogous with the already established structures of other L-asparaginase proteins. Docking result showed the formation of 7 hydrogen bonds and the interactions were occurring at ASP138, THR137, SER163, ASP105, THR32 & SER106 at the predicted active with good binding energy which indicates that the ligand-asparagine will show good activity with the target L-asparaginase protein. The present study gives base line information on the homology model of fungal L-asparaginase from *F. solani* CLR-36 and its interaction with L-asparagine substrate which may provide a reliable model which could be used in the designing of alternative useful drug in the chemotherapy of ALL.

5. REFERENCES

1. Jha SK, Pasrija D, Sinha RK, Singh HR, et al. *International Journal of Pharmaceutical Sciences and Research*, 2012; **3**:3076-3090.
2. Piątkowska-Jakubas B, Krawczyk-Kuliś M, Giebel S, Adamczyk-Cioch M, et al. *Polskie Archiwum Medycyny Wewnętrznej*, 2008; **118**:664-668.
3. Senthil K M, Selvam K, Singaravel R. *International Journal of Research in Biotechnology and Biochemistry*, 2012; **2**:1-12.

4. Offman MN, Krol M, Patel N. *Blood*, 2011; **117**:1614-1621.
5. Lubkowski J, Wlodawer A, Housset D, Weber IT, et al. *Acta Crys-tallogr D Biol Crystallogr*, 1994; **50**:826-832.
6. Neuman RE, McCoy TA. *Science*, 1956; **124**:124-125.
7. Talluri VP, Bhavana M, Mahesh Kumar MVS, Rajagopal SV. *International Letters of Natural Sciences*, 2014; **15**:23-35.
8. Godfrin Y, Bertrand Y. *BioMedES*, 2006; **1**:10-13.
9. Aghaiypour K, Wlodawer A, Lubkowski J. *Biochemistry*, 2001; **40**:5655-5664.
10. Klumper E et al. *Blood*, 1995; **86**:3861-3868.
11. Reddy ER, Babu RS, Chandrasai PD, Madhuri P. *Biotech*, 2016; **6**:105.
12. Greenberg DM, Blumenthal G, Ramadan MA. *Cancer Res*, 1964; **24**:957–963.
13. Bhosale H, As-Suhbani AE. *Journal of Experimental Biology and Agricultural Sciences*, 2019; **7**:396-402.
14. As-Suhbani AE, Bhosale H. *International Journal of Pharmacy and Pharmaceutical Research*, 2019; **15**:277-292
15. Marti-Renom MA, Stuart A, Fiser A, Sanchez R, Melo F, et al. *Annu. Rev. Biophys. Biomol. Struct*, 2000; **29**:291-325.
16. Sali A, Blundell TL. *J. Mol. Biol*, 1993; **234**:779-815.
17. Fiser A, Do RK, Sali A. *Protein Science*, 2000; **9**:1753-1773.
18. Morris GM, Goodsell D S, Halliday RS, Huey R, et al. *J. Comput. Chem*, 1998; **19**:1639-1662.
19. Ortlund E, Lacount MW, Lewinski K, Lebioda L. *Biochemistry*, 2000; **39**:1199-204.
20. Palm GJ, Lubkowski J, Derst C, Schleper S, Röhm KH, Wlodawer A. *FEBS Lett*, 1996; **390(2)**:211-216.
21. BIOVIA DS. Discovery Studio Visualizer, San Diego, Dassault Systemes, 2015.