

Original Research Article

Modeling and validation of L-asparaginase enzyme, an anticancer agent using the tools of computational biology

Praveen Reddy P.*

Department of Microbiology, Vivekananda Degree and PG College, Karimnagar, Telangana, India

Received: 25 October 2019

Revised: 18 November 2019

Accepted: 03 December 2019

***Correspondence:**

Dr. Praveen Reddy P.,

E-mail: microprr@gmail.com

Copyright: © the author(s), publisher and licensee Medip Academy. This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License, which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

ABSTRACT

Background: The L-Asparaginase is a medically important drug. The L-Asparaginase enzyme, an anticancer agent produced by microorganisms is used for the treatment of patients suffering from lymphoma and leukemia. The L-Asparaginase is economical and its administration is easy when compared to other commercial drugs available in market. Many microbes have been reported to produce the L-Asparaginase.

Methods: In the present work the sequence of L-Asparaginase enzyme protein was obtained from the Universal Protein Resource (UNIPROT) server. The sequence of L-Asparaginase was used to generate 3-D model of L-Asparaginase in SWISS MODEL server. The constructed L-Asparaginase model was verified using Ramachandran Plot in PROCHECK server.

Results: The FASTA format of L-Asparaginase enzyme of *Bacillus subtilis* strain 168 was retrieved from UNIPROT server. The FASTA format of L-Asparaginase was submitted to SWISS MODEL and its three-dimensional structural model was developed based on relevant template model. The model structure of L-Asparaginase was validated in PROCHECK server using Ramachandran Plot. The Ramachandran Plot of L-Asparaginase model inferred the reliability of L-Asparaginase structure model developed in SWISS MODEL server.

Conclusions: In the present study computational tools were exploited to develop and validate a potent anticancer drug, L-Asparaginase. Further the modeled L-Asparaginase enzyme protein can be improved using advanced bioinformatics tools and the same improved enzyme can be produced by improving the L-Asparaginase producing microbial strains by site-directed mutagenesis in the corresponding gene.

Keywords: Anticancer, L-Asparaginase, PROCHECK server, SWISS MODEL server, Universal protein resource server

INTRODUCTION

The L-Asparaginase of microbial origin is a medically important enzyme as it can be employed for the treatment of different types of human cancers, especially, lymphoma and leukemia.¹ The L-Asparaginase enzyme is easily obtained from various microbes. It is economical and more convenient to use for the treatment of cancer patients when compared to costly drugs available in the market.² In healthy humans the L-Asparagine amino acid is produced in the cells by the activity of L-Asparagine

synthetase. The L-Asparagine also occurs in regular human diet. In cancer patients' normal cells can synthesize L-Asparagine but tumour cells cannot as the enzyme, L-Asparagine synthetase is inactive in them. Hence, tumour cells depend upon the nutrient sources which are transported through blood after digestion.

When L-Asparaginase is injected into a patient it degrades L-Asparagine into aspartic acid and ammonia in blood and thus making L-Asparagine not available to tumour cells. This leads to the death of tumour cells.³⁻⁵

In the present scenario computational biology tools have become very significant in the area of biological research. These computational tools can be integrated with the experiments of molecular biology and structural biology for the generation of improved biomolecules. In the present paper the L-Asparaginase enzyme protein sequence obtained in UNIPROT database was analyzed in SWISS MODEL server by automated mode to generate the corresponding protein model. The quality of the modeled L-Asparaginase enzyme protein was validated in PROCHECK.

METHODS

Deriving L-Asparaginase enzyme sequence

The L-Asparaginase enzyme protein sequence of microbial (bacterial) was obtained in UNIPROT (www.uniprot.org). The UNIPROT is a web-based server which includes the protein sequences of various databases.⁶ The UNIPROT is composed of three types of databases viz., UniProt Knowledgebase, UniProt Archive and UniProt Reference clusters. The UniProt Archive is a bank of sequences from which a desired protein sequence can be procured. The information related to proteins can be retrieved from UNIPROT server by entering the name of living organism and its protein.

Modeling of L-Asparaginase enzyme protein structure in SWISS MODEL server

The L-Asparaginase enzyme protein sequence was used to develop the corresponding L-Asparaginase 3-D model in SWISS MODEL server. The SWISS MODEL provides an environment for users to build protein models. The amino acid sequence of a protein is sufficient to obtain the corresponding model of protein in SWISS MODEL workspace. The modeling of a protein in SWISS MODEL server includes entry of protein sequence, searching of template, selection of template, construction of three-dimensional model of protein and model validation. The L-Asparaginase enzyme sequence was submitted to SWISS MODEL server to obtain the three-dimensional structure of L-Asparaginase model based on best matching template protein. In SWISS MODEL server a protein model can be generated by automated mode.⁷

Determination of model quality of developed L-Asparaginase enzyme protein

The model quality was checked using Ramachandran plot in PROCHECK. The PROCHECK is a server which allows the users to validate protein models online.⁸ The PROCHECK determines the quality of a protein model. The PDB format of a protein has to be entered in the PROCHECK server to validate the protein model. The server checks the protein quality by Nuclear Magnetic Resonance (NMR). A Ramachandran plot is generated in PROCHECK for a submitted protein model. Based on the

percentage of amino acids in various regions viz., favored, additional allowed, generously allowed and disallowed regions in Ramachandran plot the quality of the protein is determined. The present work was performed between August 2019 to September 2019 and for the present work inclusion and exclusion criteria is not applicable.

RESULTS

Amino acid sequence of L-Asparaginase enzyme protein collected from UNIPROT

The following amino acid sequence of L-Asparaginase (L-Asparaginase 1) of *Bacillus subtilis* strain 168 was obtained from UNIPROT.

MKKLLMLTTGGTIASVEGENGLAPGVKADELLSY
 VSKLDNDYTMETQSLMNIDSTNMQPEYWVEIAEA
 VKENYDAYDGFVITHGTDITMAYTSAALS YMLQHA
 KKPIVITGSQIPITFQKTD AKKNITDAIRFACEGVGG
 VYVVFDRVIQGTTRAIKLRTKSYDAFESINYPYIAFI
 NEDGIEYNKQVTEPENDTFTVDTSLCTDVCLLKLH
 PGLKPEMFDALKSMYKGVIESYGGGVPFEGRDIL
 SKVNELIESGIVVVITTCLEEGEDMSIYEVGRRVN
 QDLIIRSRNMNTEAIVPKLMWALGQSSDLPVVKRI
 METPIADDVVLL

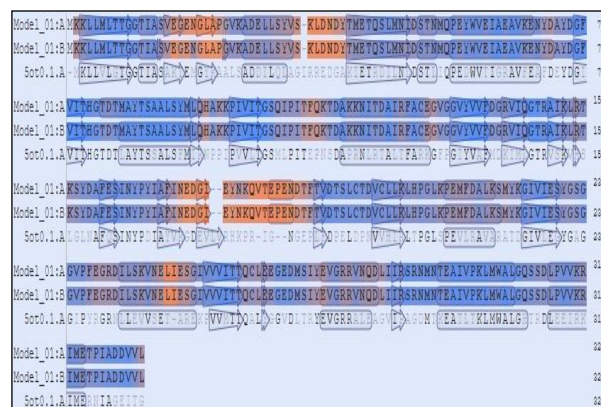


Figure 1: Alignment of sequence of L-Asparaginase enzyme protein with its template, 5ot0.1.A sequence.

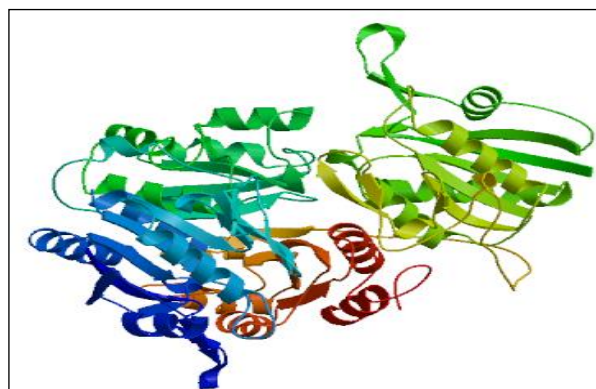


Figure 2: L-Asparaginase enzyme model.

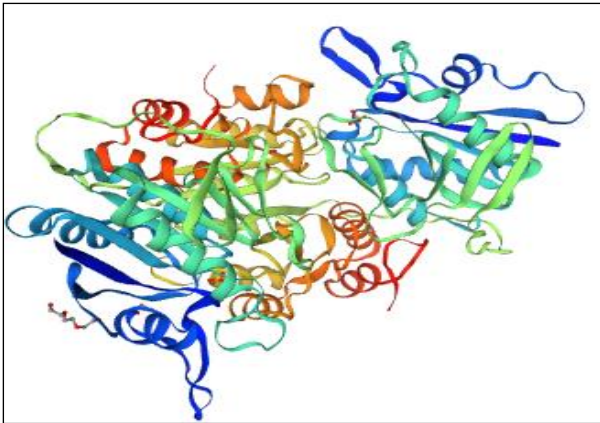
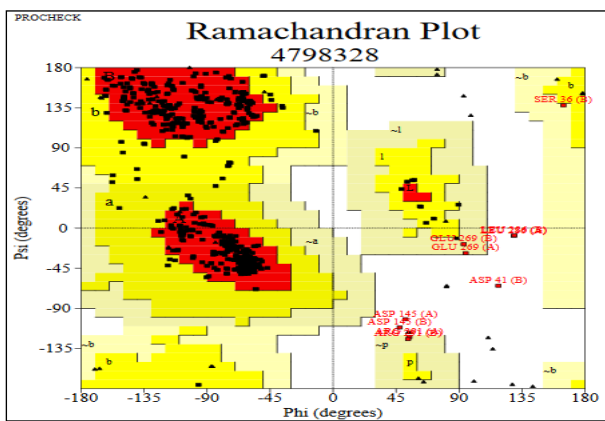


Figure 3: Template (5ot0.1.A) model.



Symbols/Alphabets indication:
 A, B, L – Amino acids in favored regions
 a, b, l, p – Amino acids in additional allowed regions
 ~a, ~b, ~l, ~p-Amino acids in generously allowed regions

Figure 4: Ramachandran plot of modeled L-Asparaginase enzyme

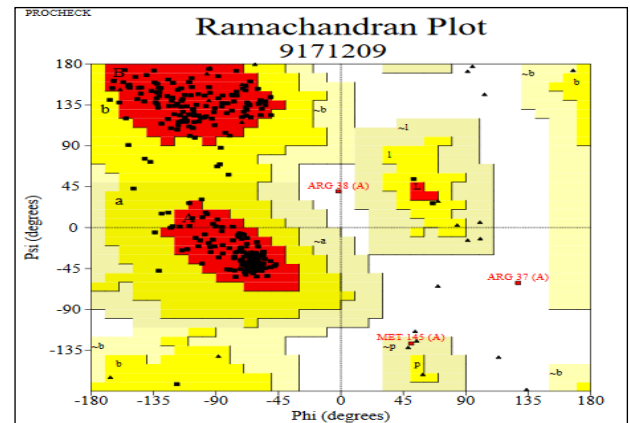
Generation of model of L-Asparaginase enzyme protein in SWISS MODEL

The model of L-Asparaginase enzyme protein was developed in SWISS MODEL by automated mode based

on its amino acid sequence. The given sequence of protein (L-Asparaginase) aligns with various template protein sequences in SWISS MODEL. The template exhibiting the maximum similarity was used to build the L-Asparaginase enzyme model. The best matching template was found to be 5ot0.1. A. The alignment of L-Asparaginase sequence and template (5ot0.1. A) sequence is depicted in Figure-1. The model of L-Asparaginase enzyme (Figure-2) was obtained using the template, 5ot0.1. A model (Figure-3).

Validation of modeled L-Asparaginase enzyme protein

The models of L-Asparaginase enzyme protein and its template were validated in PROCHECK server using Ramachandran plots. The Ramachandran plots of both modeled L-Asparaginase enzyme protein (Figure-4) and template (Figure 5) were generated. The percentage of amino acid residues (more than 90%) in most favored regions (Table 1) implied the genuine quality of L-Asparaginase and template models.



Symbols/Alphabets indication:
 A, B, L – Amino acids in Favored regions
 a, b, l, p – Amino acids in Additional allowed regions
 ~a, ~b, ~l, ~p-Amino acids in generously allowed regions

Figure 5: Ramachandran plot of template (5ot0.1.A) model.

Table 1: Data of Ramachandran plots of modeled L-Asparaginase enzyme and its template.

Sl. No.	Protein model	Percentage of amino acid residues in most favoured regions	Percentage of amino acid residues in additional allowed regions	Percentage of amino acid residues in generously allowed regions	Percentage of amino acids in disallowed regions
1.	L-Asparaginase model	90.5%	7.8%	0.9%	0.9%
2.	Template (5ot0.1. A) model	92.0%	6.9%	0.4%	0.7%

DISCUSSION

In the present work, the model of L-Asparaginase enzyme protein was generated in SWISS MODEL

WORKSPACE by automated mode using the corresponding amino acid sequence i.e., its primary protein sequence collected from UNIPROT. The

UNIPROT is composed of publicly accessible non-repeated sequences of proteins available in various protein databases.⁶ The L-Asparaginase enzyme is a tetramer made up of four identical monomers viz., A, B, C and D chains. Actually it can be considered as dimer of dimers. The monomers, A and C intimately associate to form an intact dimer. Similarly, monomers B and D closely associate to form a dimer. Both these dimers interact to form a tetramer of L-Asparaginase.⁹ For the primary sequence of L-Asparaginase enzyme 66 templates were obtained in STML of SWISS MODEL.

The template exhibiting highest similarity to the L-Asparaginase was 5ot0.1.A, based on which, model of L-Asparaginase enzyme protein was built. The sequences of A and B monomers of L-Asparaginase enzyme were matched with the template, 5ot0.1.A sequence during modeling. The SWISS MODEL is an environment which facilitates the researchers to generate protein models. The FASTA format of protein sequence is needed to be entered into the SWISS MODEL server to generate the respective protein model by automated mode. In SWISS MODEL the submitted primary protein sequence is matched with template sequences present in STML, the template library. The template with maximum similarity is used to develop the given protein model.¹⁰ The L-Asparaginase enzyme protein and its template models were validated in PROCHECK by Ramachandran plots. Above 90% of amino acid residues in Ramachandran plots of both L-Asparaginase model and its template were in most favored regions inferring the good quality of their models.^{8, 11}

CONCLUSION

In the present work the L-Asparaginase enzyme protein sequence was retrieved from UNIPROT server and its model structure is built in SWISS MODEL. The developed L-Asparaginase model was validated by Ramachandran plot in PROCHECK. The present work emphasizes on the exploitation of web based computational tools in biological research. The modeled and validated L-Asparaginase enzyme can be further improved using bioinformatics software tools like 'Discovery Studio' by modifying the amino acids at specific sites. The corresponding changes can be made in the L-Asparaginase gene of microbes to synthesize L-Asparaginase with enhanced activity.

ACKNOWLEDGEMENTS

The author express thanks to Correspondent, Principal and Administrative officer, Vivekananda Degree and PG College, Karimnagar, Telangana, India for their support and encouragement during the completion of present work. The author extend thanks to Head, Department of Computer Science for permitting to use the computer lab.

Funding: No funding sources

Conflict of interest: None declared

Ethical approval: Not required

REFERENCES

1. Mangamuri U, Vijayalakshmi M, Ganduri VS, Rajulapati SB, Poda S. Extracellular L-Asparaginase from *Streptomyces labedae* VSM-6: Isolation, Production and Optimization of Culture Conditions Using RSM. *Pharmacog J.* 2017;9(6).
2. Kamble KD, Bidwe PR, Muley VY, Kamble LH, Bhadange DG, Musaddiq M. Characterization of L-asparaginase producing bacteria from water, farm and saline soil. *Biosci Discov.* 2012;3(1):116-9.
3. Yogendra S, Srivastava SK. Isolation and Characterization of Microorganisms capable of producing antineoplastic drug, L-asparaginase. *International J Biol Med Res.* 2012;3:2548-54.
4. Bhat MR, Nair JS, Marar T. Isolation and identification of L-asparaginase producing *Salinicoccus* sp. M KJ997975 from soil microbial flora. *Intern J Pharmaceutical Sci Res.* 2015;6(8):3599.
5. Jaya Kumar A, Balakrishna Pillai A, Kumarapillai H. Sequencing and characterization of L-asparaginase (ansB) gene of *Bacillus megaterium* isolated from Western Ghats, Kerala, India. *Intern J Current Microbiol Applied Sci.* 2015;4(6):753-60.
6. Apweiler R, Bairoch A, Wu CH, Barker WC, Boeckmann B, Ferro S, et al. UniProt: the universal protein knowledgebase. *Nucleic Acids Res.* 2004;32(1):D115-9.
7. Arnold K, Bordoli L, Kopp J, Schwede T. The SWISS-MODEL workspace: a web-based environment for protein structure homology modelling. *Bioinformatics.* 2006;22(2):195-201.
8. Balaji S, Kalpana R, Shapshak P. Paradigm development: comparative and predictive 3D modeling of HIV-1 Virion Infectivity Factor (Vif). *Bioinformation.* 2006;1(8):290.
9. Yun MK, Nourse A, White SW, Rock CO, Heath RJ. Crystal structure and allosteric regulation of the cytoplasmic *Escherichia coli*-asparaginase I. *J Mole Biol.* 2007;369(3):794-811.
10. Waterhouse A, Bertoni M, Bienert S, Studer G, Tauriello G, Gumienny R, et al. SWISS-MODEL: homology modelling of protein structures and complexes. *Nucleic Acids Res.* 2018;46(W1):W296-303.
11. Laskowski RA, MacArthur MW, Moss DS, Thornton JM. PROCHECK: a program to check the stereochemical quality of protein structures. *J Applied Crystallo.* 1993;26(2):283-91.

Cite this article as: Reddy PP. Modeling and validation of L-asparaginase enzyme, an anticancer agent using the tools of computational biology. *Int J Res Med Sci* 2020;8:211-4.