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IN-SILICO MODELING AND VALIDATION OF L-GLUTAMINASE ENZYME, AN ANTICANCER DRUG USING WEB-BASED COMPUTATIONAL TOOLS

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ABSTRACT: The L-Glutaminase enzyme is a therapeutic agent that can be employed for the treatment of human cancer, specifically lymphocytic leukemia. Microbes are exploited commercially to produce L-Glutaminase on a large scale. The L-Glutaminase is an economic anti-cancer drug and can be easily administered into patients. The L-Glutaminase is also employed in food industries in the processing of fermented foods. In the present paper, the L-Glutaminase enzyme structure was developed and validated using web-based computational tools. The sequence (FASTA format) of the L-Glutaminase enzyme was obtained from UniProt Knowledgebase. The FASTA format sequence of the L-Glutaminase enzyme was used as input data in the SWISS MODEL workspace to develop the structural model of L-Glutaminase by automated mode. The quality of the modeled structure of the L-Glutaminase enzyme was checked in PROCHECK server and SPDBV (Swiss PDB Viewer) and validated. Further, L-Glutaminase activity can be enhanced using advanced bioinformatics tools. Such L-Glutaminase with improved activity can be produced by microorganisms.

INTRODUCTION: The L-Glutaminase enzyme produced by microbes has gained medical importance as it can be used for the treatment of certain types of human cancers, especially, lymphocytic leukemia. L-Glutaminase production is economical, and its usage is eco-friendly. Its cost is very low when compared to expensive commercial drugs. The L-Glutamine is an important amino acid for the living cells. It is available in the regular diet, and even it is synthesized by the living cells by the catalytic activity of L-glutamine synthetase.

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In cancer cells, the activity of L-glutamine synthetase is ceased, and thus they depend upon the L-Glutamine that is transported through blood (digested food nutrient). In the blood of leukemia patients, L-Glutaminase converts L-Glutamine into L-Glutamic acid and ammonia. Hence, L-Glutamine is not available to cancer cells, and eventually, they die. In addition, L-Glutaminase is used in the food industry in the preparation of fermented foods like soy sauce [1-3].

Proteins are important biomolecules as they perform various functions in living cells. Some microbial proteins are commercially important, especially proteins with therapeutic value and industrial importance. Developing a protein structure by performing experiments is a laborious and time-consuming process. On the basis of primary structure, *i.e.*, amino acid sequence, the structure of a protein can be generated using

bioinformatics software tools. Many of the protein modeling software tools are available as web-based tools [4]. The various web-based computational tools available for protein modeling are SWISS-MODEL workspace, 3-D JIGSAW, PHYRE, PUFUE, THREADER, mGENThreader, *etc*.

In the present work, the amino acid sequence of L-Glutaminase enzyme protein in FASTA format derived from the UniProt database server was used to develop L-Glutaminase structural model in SWISS-MODEL workspace. Then model quality was checked in PROCHECK and SPDBV (Swiss PDB Viewer) and validated.

MATERIALS AND METHODS:

Collection of Amino acid Sequence of L-Glutaminase Enzyme Protein: The amino acid sequence of L-Glutaminase enzyme was obtained from UniProt server. The UniProt Knowledgebase is a collection of protein sequences from various protein databases. The stored protein sequences of different organisms can be retrieved by users [5].

Homology Modeling of L-Glutaminase Enzyme Protein: The FASTA format of L-Glutaminase enzyme protein sequence was used to develop the corresponding structural model by automated mode in SWISS-MODEL workspace. The SWISS-MODEL workspace is a user-friendly server that needs the input of an amino acid sequence of protein in FASTA format to build a corresponding protein model [6].

Verification of Model Quality of the L-Glutaminase Enzyme Protein Model: The modeled structure of L-Glutaminase enzyme protein was checked in PROCHECK using Ramachandran plot [7] and based on RMSD (root mean square deviation) value obtained between main-chain atoms of L-glutaminase model and its template model in SPDBV [8].

RESULTS AND DISCUSSION:

Sequence of L-Glutaminase Enzyme Protein: The amino acid sequence of L-Glutaminase enzyme protein (L-Glutaminase 2) of *Escherichia coli* strain K12 was retrieved from the UniProt protein database server. The FASTA format of sequence is shown below.

MAVAMDNAILENILRQVRPLIGQGKVADYIP
ALATVDGSRLGIAICTVDGQLFQAGDAQERF
SIQSISKVLSLVVAMRHYSEEEIWQRVGKDPS
GSPFNSLVQLEMEQGIPRNPFINAGALVVCD
MLQGRLSAPRQRMLEVVRGLSGVSDISYDTV
VARSEFEHSARNAAIAWLMKSFGNFHHDVTT
VLQNYFHYCALKMSCVELARTFVFLANQGK
AIHIDEPVVTPMQARQINALMATSGMYQNAG
EFAWRVGLPAKSGVGGGIVAIVPHEMAIAVW
SPELDDAGNSLAGIAVLEQLTKQLGRSVY

The L-Glutaminase enzyme is composed of 308 amino acids. UniProt Knowledgebase is a protein resource database which is composed of sequential, structural, and functional information of proteins. Users can retrieve desired protein sequences in FASTA format. Desai *et al.,* obtained the amino acid sequence (FASTA format) of protein X of Hepatitis B virus from the UniProt database and its 3-D structure was modeled in Phyre 2 [9].

Modeling of L-Glutaminase Enzyme Protein Structure: The FASTA format sequence of L-Glutaminase enzyme protein was used to develop a structural model of L-Glutaminase by automated mode in SWISS-MODEL workspace. The SWISS-MODEL is a web-based tool in which a protein structure is modeled based on the amino acid sequence in FASTA format. The given sequence, *i.e.,* the query amino acid sequence of the protein, is aligned and matched with sequences of proteins present in the data bank of SWISS-MODEL. The protein sequence of the SWISS-MODEL protein databank, which shows the closest similarity to the query protein sequence, is regarded as a template based on which the model of the target protein is developed [10]. In SWISS-MODEL workspace the template, 1u60.1.A showed maximum similarity to the L-Glutaminase sequence and based on which structure model of the L-Glutaminase enzyme protein was built. The L-Glutaminase enzyme is a tetramer made up of four identical subunits (poypeptide chains). The L-Glutaminase enzyme exits as dimer and tetramer in the oligomeric state.

The tetramer form of L-Glutaminase is formed by the union of two dimers. The monomers are intimately associated in each dimer. Thus, the tetramer can be regarded as a dimer of dimers [11]. In SWISS-MODEL, the sequences of two homodimers (identical chains A and B) of L-Glutaminase enzyme protein were aligned with the template, 1u60.1.A sequence **Fig. 1**. The

homodimer form of the L-Glutaminase enzyme protein model **Fig. 2** with two identical subunits (A and B) was generated based on the template (1u60.1.A) model **Fig. 3**. Ghosh *et al.*, [12] generated the modeled structure of freshwater fish ATPases in the SWISS-MODEL workspace.

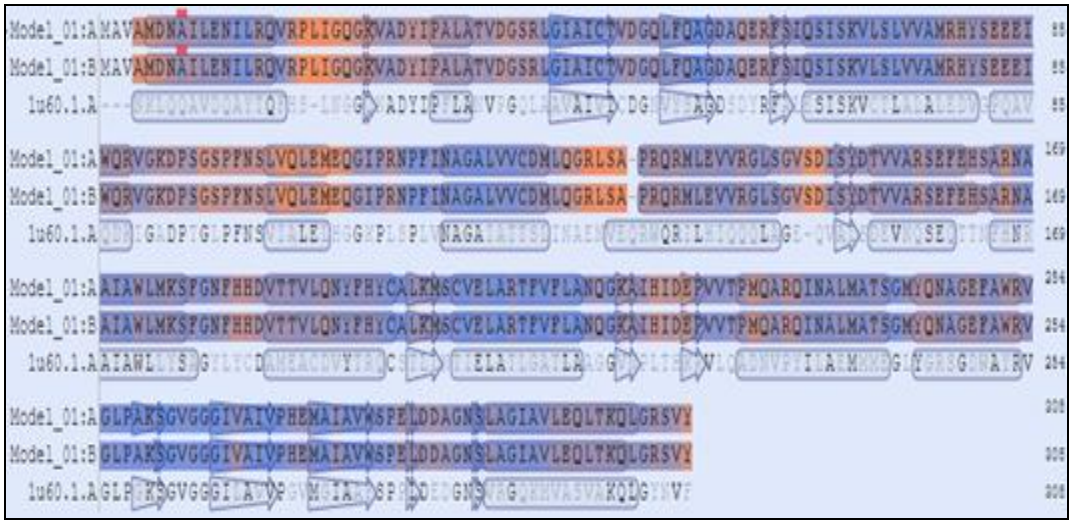

FIG. 1: ALIGNMENT OF SEQUENCES OF TWO CHAINS, A AND B (HOMODIMER) OF L-GLUTAMINASE ENZYME WITH THE TEMPLATE, 1u60.1.A SEQUENCE

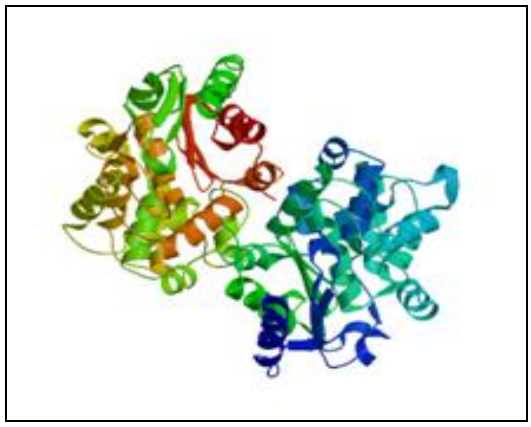

FIG. 2: L-GLUTAMINASE MODEL (HOMODIMER FORM)

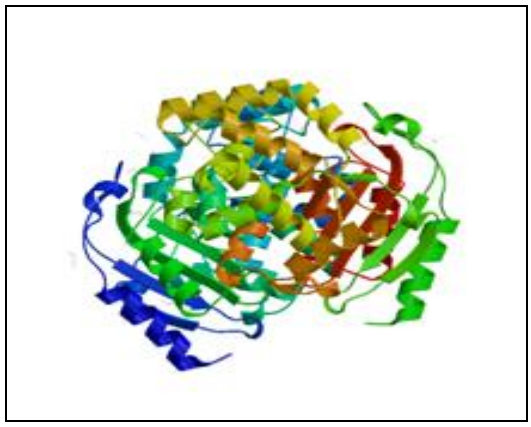

FIG. 3: TEMPLATE (1u60.1.A)

Validation of Modeled L-Glutaminase Enzyme Protein Structure: The model quality of the L-Glutaminase enzyme and its template, 1u60.1.A was determined in PROCHECK based on the percentage of amino acid residues found in most favored, additionally allowed, generously allowed and disallowed regions. In both the Ramachandran plots of L-Glutaminase **Fig. 4** and template **Fig. 5** models above 90% of the amino acids were observed in most favored regions **Table 1,** indicating the reliability of the quality of L-Glutaminase and its template models. As per PROCHECK protein quality standard, the quality of a protein model is good if, in its Ramachandran plot above 90% of the amino acids are in the most favored region [13]. Moholkar *et al.* [14] determined model quality of E1 structural glycoprotein by using the Ramachandran plot in PROCHECK.

Further, the quality of the L-Glutaminase enzyme model was validated in SPDBV. The SPDBV is a software tool that allows the users to superimpose the target protein over its template to calculate the RMSD. Based on the RMSD value, the quality of the modeled protein structure is determined. The main chain atoms of the L-Glutaminase model were superimposed on its template model **Fig. 6,** and the RMSD value was calculated. The RMSD value obtained was very low (0.34 A°), ensuring the good quality of the modeled L-Glutaminase structure. Kankate and Nathe [15] validated model quality of lanosterol-1,4-alpaha- demethylase enzyme structure based on the low RMSD value obtained by superimposing its model on the corresponding template in SPDBV.

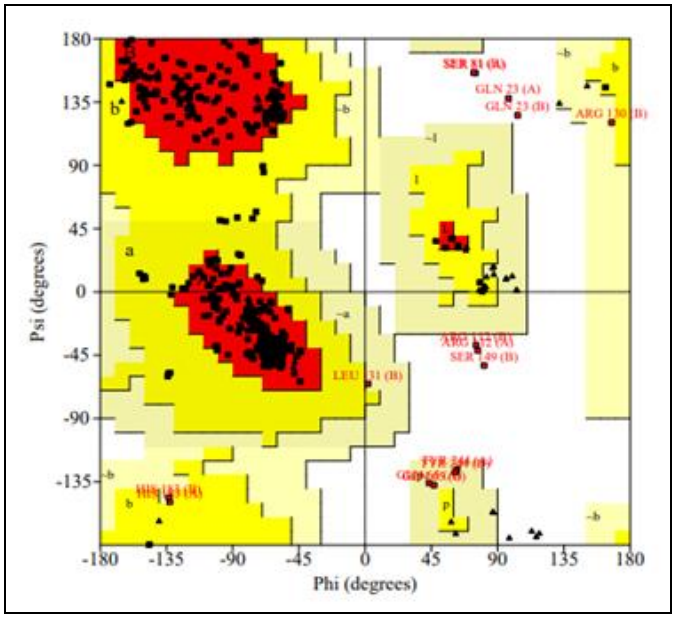

FIG 4: RAMACHANDRAN PLOT OF MODELED L-GLUTAMINASE ENZYME GENERATED IN PROCHECK. A, B, L: Amino acids in favored regions. a, b, l, p: Amino acids in additional allowed regions. ~a, ~b, ~l, ~p: Amino acids in generously allowed regions

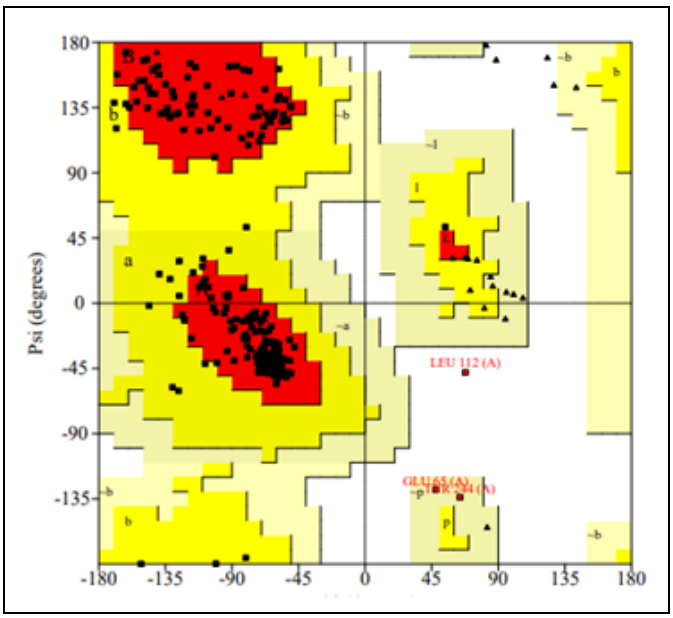

FIG. 5: RAMACHANDRAN PLOT OF TEMPLATE (1u60.1.A) GENERATED IN PROCHECK. A, B, L: Amino acids in favored regions. a, b, l, p: Amino acids in additional allowed regions. ~a, ~b, ~l, ~p: Amino acids in generously allowed regions

TABLE 1: PERCENTAGE OF AMINO ACIDS IN DIFFERENT REGIONS OF RAMACHANDRAN PLOTS OF L-GLUTAMINASE AND TEMPLATE MODELS

S. no.	Protein model	Amino acids in most favored regions (A, B, L)	Amino acids in additionally allowed regions (a, b, l, p)	Amino acids in generously allowed regions (~a, ~b, ~l, ~p)	Amino acids in disallowed regions
1	L-Glutaminase	91.5%	5.6%	1.5%	1.3%
2	Template(1u60.1.A)	90.5%	8.3%	0.8%	0.4%

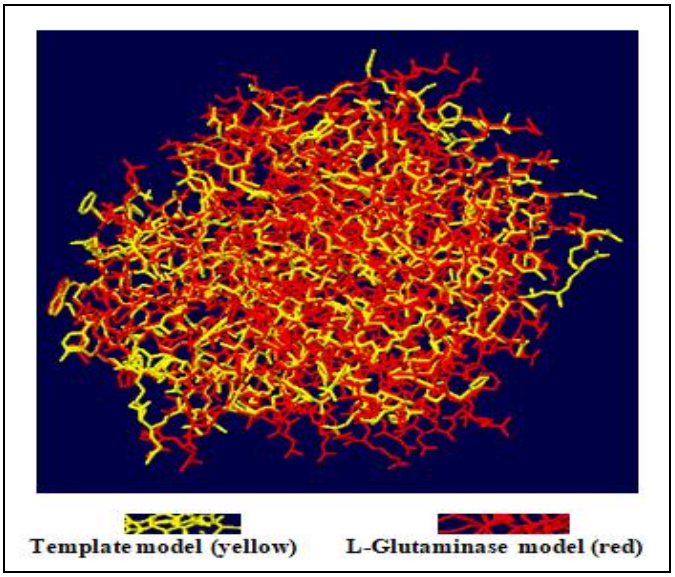

FIG. 6: SUPERIMPOSITION OF L-GLUTAMINASE ENZYME AND TEMPLATE (1u60.1.A) MODELS IN SPDBV

CONCLUSION: In the present study sequence of the L-Glutaminase enzyme obtained from UniProt was used to build a structural model of L-Glutaminase in SWISS-MODEL workspace by automated. Then model quality was validated in PROCHECK using Ramachandran plots and based on RMSD value calculated between the main chain atoms of L-Glutaminase model and its template, 1u60.1. An in SPDBV. Further, the activity of L-Glutaminase can be improved by changing amino acids at specific sites using advanced bioinformatics software tools. The improved L-Glutaminase enzyme can be synthesized from microbes by making the corresponding modifications in the nucleotides of the L-Glutaminase gene.

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CONFLICTS OF INTEREST: The author declares that there are no conflicts of interest.

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