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IN SILICO MODELING AND VALIDATION OF BACTERIAL RIBONUCLEASE (BINASE), AN ANTI-CANCER AGENT

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ABSTRACT

The ribonucleases perform various cellular functions. In addition, they exhibit certain therapeutic properties. Especially, the bacterial ribonuclease (binase) is a potent anti-cancer and antiviral agent. In the present paper, the sequence of binase enzyme was collected from UniProt web-server, based on which binase enzyme model (three-dimensional) was built in Phyre2 server. The model quality of binase enzyme was evaluated and validated using Verify3D and PROCHECK servers. Further the activity of binase can be enhanced using sophisticated computational tools. Such binase enzyme with improved activity can be produced in the laboratory by making corresponding changes in binase gene through site-directed mutagenesis in microbes.

Key words: Ribonuclease, Binase, Anti-cancer, Binase enzyme model, Model quality

INTRODUCTION

The Ribonucleases (RNases) perform various vital functions in living cells. They are involved in DNA replication, defense mechanism, regulation of ribonucleic acid (RNA) metabolism and expression of protein coding genes. The RNases are majorly categorized into endo- and exo-ribonucleases. The endo-ribonucleases break

RNA in 5' - 3' direction whereas exo-ribonucleases cleave in 3' - 5 ' direction [1].

In addition to cellular functions, RNases exhibit anticancer and antiviral property. They show antitumor property by destroying the RNA of cancer cells. A combination of RNases with other anticancer agents was proved to be an effective therapeutic preparation. Various RNases with potent therapeutic properties include BS-RNase (*Bos Taurus*), Onconase (*Rana pipiens*), Binase (*Bacillus intermidius*), RNase7 (*Homo sapiens*), Angiogenin (*Homo sapiens, Mus musculus* and *Rattus norvegicus*) and RNase2 (*Homo sapiens*) [1, 2].

The RNase (binase) produced by bacterial species exhibited potent anticancer properties. The bacterial species which produce binase include *Bacillus amyloliquefaciens, Streptomyces aureofaciens* and *Bacillus intermedius*. The binase produced by *Bacillus intermedius* is very effective and does not require any cofactor for its activity. The binase was the first detected antitumor RNase of bacterial origin. It exhibited significant cytotoxic activity against cancer cells. In addition, bacterial binase is less immunogenic. The binase was also proved to be an active antiviral agent against rabies and influenza virus [3, 4].

Numerous gene and protein databanks are available online from which users can freely obtain the desired gene and protein sequences. Computational tools are extensively used to construct and evaluate protein models. The protein modeling is a developing area in the field of bioinformatics. The scientists from engineering, physical and biological sciences had combined their thoughts to design novel software programs (Bioinformatics tools) to generate and enhance protein models. Currently, various online bioinformatics tools are available and they are easy to use without any previous programming knowledge. [5].

The present study was planned to generate and validate the three-dimensional model of bacterial Ribonuclease (binase) using bioinformatics tools based on its amino acid sequence retrieved from UniProt database server. For Ribonuclease (binase) modeling Phyre2 server was used. The model quality of binase 3-D structure was determined in Verify3D and PROCHECK servers.

MATERIALS AND METHODS

Collection of amino acid sequence of binase enzyme

The amino acid sequence of binase enzyme (FASTA format) was derived from UniProt web-server which is a collection centre of various proteins databases. [6].

Construction of 3-D model of binase enzyme

The binase enzyme sequence (query sequence) was used as input data in Phyre2 server for construction of binase enzyme model structure based on a template. In Phery2, the query sequence is aligned with various protein sequences to determine the best matching sequence. The protein sequence which exhibits high degree of similarity with query sequence is considered to be a template, based on which the model of the query protein is generated [7].

Verification of binase enzyme model

The model quality of binase enzyme structure was evaluated in Verify3D and PROCHECK servers. The Verify3D and PROCHECK are the widely employed genuine web-servers for validation of protein models. In Verify3D, the model quality is validated based on overall score generated in connection with the features of amino acids present in a protein sequence. In PROCHECK, a protein model is validated by the analysis of its Ramachandran plot. [8].

RESULTS AND DISCUSSION

Binase enzyme sequence

The binase enzyme sequence of *Bacillus intermedius* was downloaded in FASTA format from UniProt server. The following

is the FASTA format sequence of binase enzyme.

MKKISSVFTMFALIAAILFSGFIPQQAYA
ETPLTQTATNETATIQLTSDVHTLAVINT
FDGVADYLIRYKRLPDNYITKSQASALG
WVASKGNLAEVAPGKSIGGDVFSNREG
RLPSASGRTWREADINYVSGFRNADRL
VYSSDWLIYKTTDHYATFTRIR

The UniProt server is a Universal Protein Knowledgebase which is formed by the union of TreEMBL, PIR protein and SwissProt databases. The UniProt is a public server from which users can easily derive the desired protein sequences [9].

Modeling of binase enzyme structure

The FASTA format of binase enzyme sequence (query sequence) was entered in Phyre2 server. In Phyre2 server, the binase enzyme sequence was matched with different protein sequences present in the Phyre2 protein databank and the protein exhibiting highest similarity was chosen as template to construct the binase enzyme model. The best matching template for binase enzyme was found to be d1goua. The alignment of d1goua and binase sequences was shown in **Figure 1**. Based on the template (d1goua) model (**Figure 2**), three-dimensional model of binase enzyme (**Figure 3**) was generated. The Phyre2 is a web-based server which is used to construct three-dimensional models

of proteins. The sequence of a protein in FASTA format is required to develop a corresponding protein model in Phery2. **[10]**.

Validation of modeled structure of binase enzyme

The model quality of binase enzyme structure generated in Phery2 was verified in most widely used model quality evaluating programs, Verify3D and PROCHECK. The PDB file of a protein is used as input in Verify3D and PROCHECK programs. The Verify3D program verifies the congruence between a protein model (3-D structure) and its amino acid sequence (primary structure/1-D). In verify3D, a score pertaining to model quality is generated based on coherence of various features of amino acids present in protein sequence (primary structure) with that of 3-D protein model. In Verify3D, for binase enzyme model, the average 3D-1D

score of 100% amino acids was >=0.2 (**Figure 4**), which inferred the best quality of binase enzyme model. In Verify3D program, to validate a protein model, minimum 80% of its amino acids average 3D-1D score must be >=0.2 **[11].** In PROCHECK server, the Ramachandran plot (**Figure 5**) pertaining to binase enzyme model was generated. In Ramachandran plot of binase model, 87.4%, 11.6%, 1.1% and 0.0% amino acid residues were distributed in most favoured, additional allowed, generously allowed and disallowed regions, respectively. The distribution of 87.4% amino acids in most favoured region (in Ramachandran plot) revealed the genuine quality of binase enzyme model. Above 80% of amino acids of a protein in most favoured region is an indication of good quality of its model **[12]**.
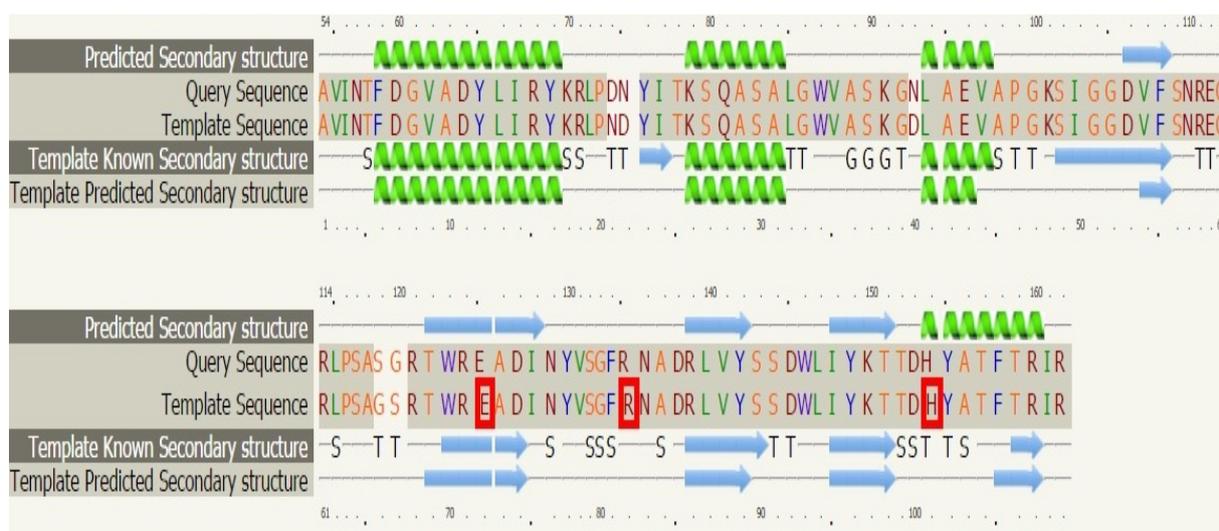
Figure 1: Alignment of binase enzyme (query sequence) and its template (d1goua) in Phyre2

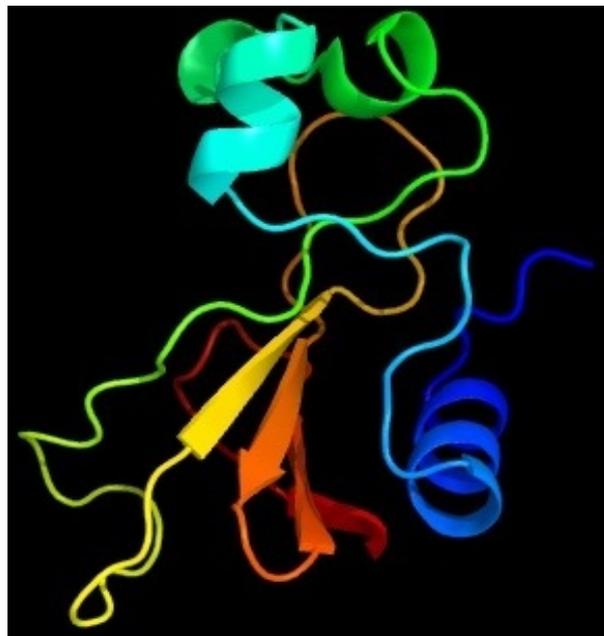**Figure 2: Template (d1goua) 3-D model**

Figure 3: Binase enzyme 3-D model

100.00% of the residues have
averaged 3D-1D score >= 0.2

Pass

At least 80% of the amino acids have scored >= 0.2 in the 3D/1D profile.

Verify3D: (ribonuclease .pdb)
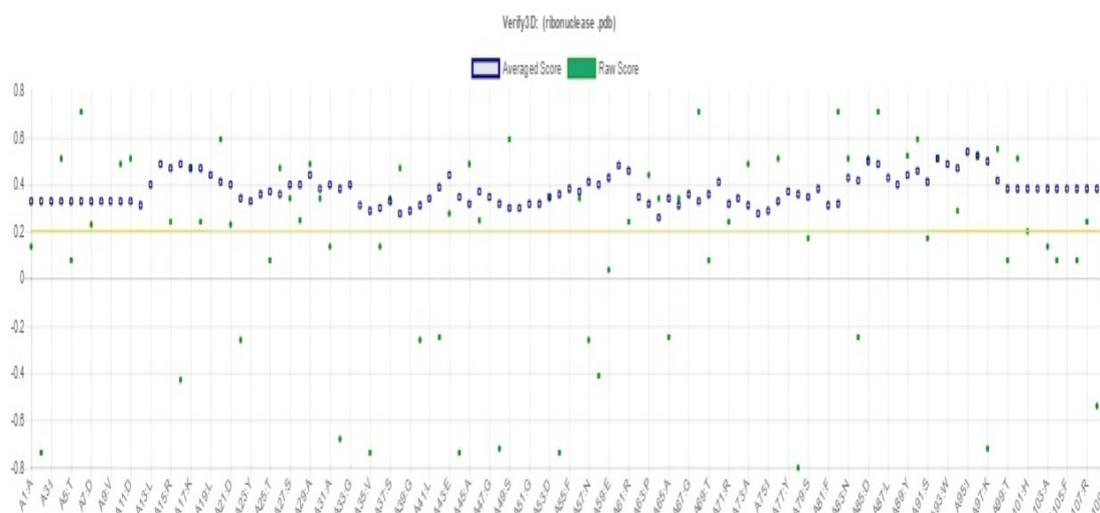
☐ Averaged Score   ■ Raw Score

Figure 4: Generation of 3D-1D score of binase enzyme model based on Amino acids organization in Verify3D program (Blue colour: Averaged score and Green Colour: Raw score)
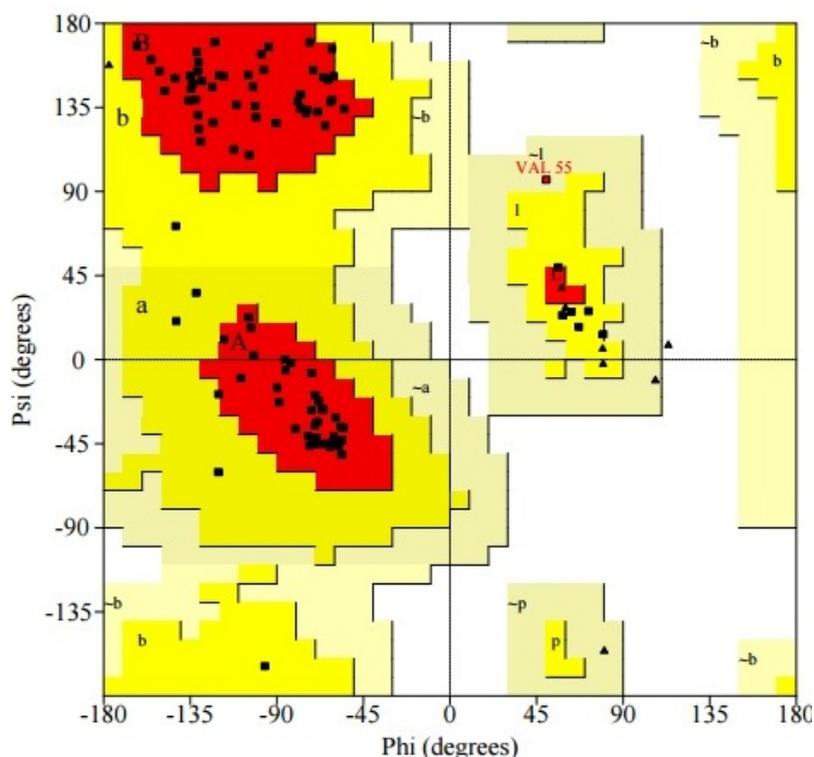

Figure 5: The Ramachandran plot of binase enzyme model generated in PROCHECK server (Red colour areas: most favoured, additional allowed and generously allowed regions; Yellow and white colour areas: disallowed regions)

CONCLUSION

In the present study, the computational tools were extensively employed to model and validate binase enzyme. The binase enzyme sequence in FASTA format retrieved from UniProt server was used to generate (Phyre2 server) and validate (Verify3D and PROCHECK servers) the binase enzyme model. The bacterial binase possess therapeutic properties in addition to its cellular functions. Such binase enzyme is medically important. Further the activity of binase can be improved using some advanced bioinformatics tools. To improve the activity, the amino acids of binase enzyme at certain specific sites can changed using software tools (like Discovery Studio) and such binase enzyme with enhanced activity can be produced by microbes through alterations in the corresponding locations of binase gene.

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