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In silico modeling and validation of uricase enzyme, an anti-gout drug

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ABSTRACT

Background

The microbial Uricase enzyme can be employed as an effective drug to cure hyperuricemia related disorders in human beings in particular gout disease and refractory gout which is developed in humans due to effects of some anti-gout synthetic drugs. Various microbes have been reported to produce Uricase. The production of Uricase enzyme by microorganisms is an economical and environmental friendly process compared to manufacturing protocols of synthetic drugs used for the same. The therapeutic enzyme, Uircase can be modified and improved by using the tools of computational biology.

Materials and methods

From UniProt Knowledgebase server the sequence of a bacterial Uricase enzyme in FASTA format was downloaded and entered in SWISS-MODEL server to develop the respective model of Uricase enzyme. The Uricase model quality was determined by using Procheck and Verify 3D online servers.

Results

The Uricase enzyme sequence of bacterium, *Arthrobacter globiformis* was procured from UniProt server. The enzyme sequence was used to develop model structure of Uricase in SWISS-MODEL server by automated model. The model quality of Uricase enzyme was checked and validated in Procheck server using Ramachandran Plot and Verify 3D server based on model quality score.

Conclusion

The bioinformatics tools can be exploited to design, model and improve the commercially important biomolecules. The proteins are the important biomolecules which can be improved by computational tools. Further the therapeutic value of Uricase enzyme can be enhanced by employing computational tools.

Keywords: Uricase enzyme, drug, gout, UniProt, SWISS-MODEL, Procheck, Verify 3D

INTRODUCTION

In human beings during purine metabolism, uric acid is formed by the oxidation of xanthine. The uric acid formed in the body is converted to allantoin by Uricase enzyme which is highly soluble and can be easily removed from blood by excretion. The Uricase enzyme is made up four identical polypeptide chains and hence it is regarded as a homotetramer. In humans, Uricase enzyme is not produced due to some non-sense and mis-sense mutations aroused during evolution of Uricase gene. This results in the accumulation of

uric acid in such human beings. The uric acid is not freely soluble and cannot be excreted easily. The accumulated uric acid results in a condition called hyperuricemia. The hyperuricemia is a clinical disorder which arises when the concentration of uric acid in serum reaches 6.8 mg/dL or more. The hyperuricemia is responsible for gout disease, hypertension, kidney failure, kidney stones and cardiovascular diseases. Hence, the Uricase enzyme plays a prominent role in the purine metabolism and excretion of the end products [1, 2]. Presently allopurinol is the drug used for the treatment of gout. But some patients are hypersensitive to allopurinol and develop refractory gout. The Uricase enzyme acts as an effective drug in the treatment of hyperuricemia, gout and even refractory gout. The continuous use of microbial (natural) Uricases is not suggestible as they may become immunogenic to humans [3].

The researchers from different sciences and engineering have combined their skills and ideas to develop software tools (bioinformatics tools). The bioinformatics tools are used to design, develop and improve biomolecules. The protein engineering is an emerging area in the field of computational biology. The proteins can be modeled and improved by employing computational tools [4].

MATERIALS AND METHODS

Uricase enzyme sequence source

The sequence of Uricase enzyme was obtained from UniProt server. The UniProt server is a Knowledgebase resource centre of proteins gathered from different protein databases. The users can obtain the sequence and functional details of a desired protein from UniProt easily [5].

In Silico building of Uricase enzyme three-dimensional model

The Uricase enzyme sequence was submitted to SWISS-MODEL to generate corresponding model of Uricase enzyme. The SWISS-MODEL is a public server which can be used freely by users. The users without prior technical knowledge can easily use SWISS-MODEL for protein modeling [6].

Determination of model quality of Uricase enzyme model

The quality of Uricase enzyme model structure was verified using Procheck and Verify 3D servers. The PDB format of Uricase was submitted to Procheck and Verify 3D servers. In these servers the Uricase model quality is checked based on the various features, properties and organization of amino acids within the model [7, 8].

RESULTS

Sequence of Uricase enzyme

The FASTA format sequence of Uricase enzyme of the bacterium, *Arthrobacter globiformis* was acquired from UniProt server. The sequence of Uricase enzyme downloaded from UniProt is given below.

MTATAETSTGTKVVLGQNQYGKAEVRLV
KVTRNTARHEIQDLNVTSQLRGDFEAAHTAG
DNAHVVATDTQKNTVYAFARDGFATTEEFLL
RLGKHFTEGFDWVTGGRWAAQQFFWDRIND
HDHAFSRNKSEVRTAVLEISGSEQAIVAGIEGL
TVLKSTGSEFHGFPRDKYTTLQETTDRILATD
VSARWRYNTVEVDFDAVYASVRGLLLKAFAE
THSLALQQTMYEMGRAVIETHPEIDEIKMSLP
NKHHFLVDLQPFGQDNPNEVFYAADRPYGLIE
ATIQREGSRADHPIWSNIAGFC

Generation of Uricase enzyme model

The sequence of Uricase enzyme in its FASTA format was introduced into SWISS-MODEL server to construct the respective model of Uricase enzyme. The Uricase enzyme sequence was compared with different protein sequences present in SWISS-MODEL to find its template protein. The template determined for Uricase enzyme was 6oe8.1.A. The alignment of (four identical sequences) of Uricase enzyme which is a homotetramer with the sequence of template is shown in figure1. Based on the template, 6oe8.1.A model (figure 2) the three-dimensional model of Uricase enzyme (figure 3) was developed.

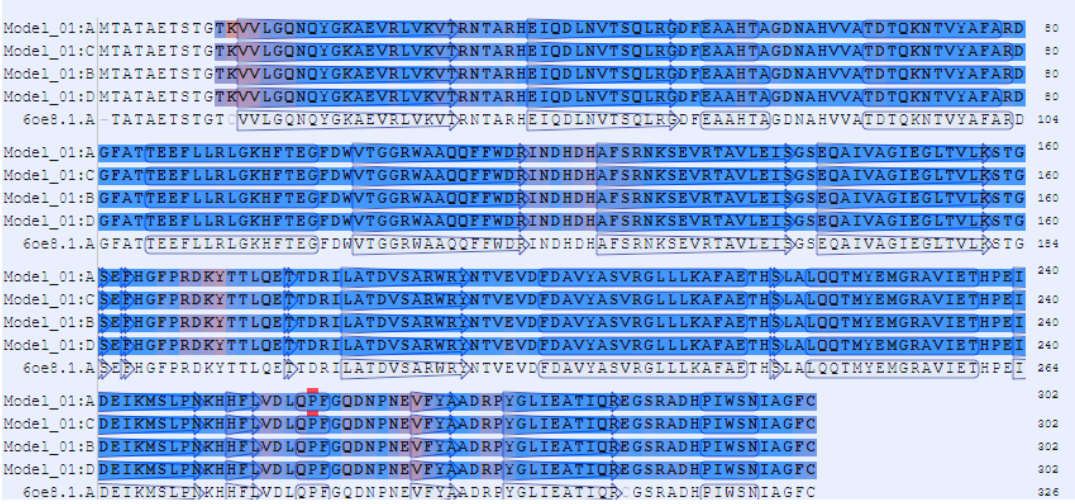

Figure 1: Alignment of Uricase enzyme sequence (sequences of homotetramers) with template, 6oe8.1.A sequence

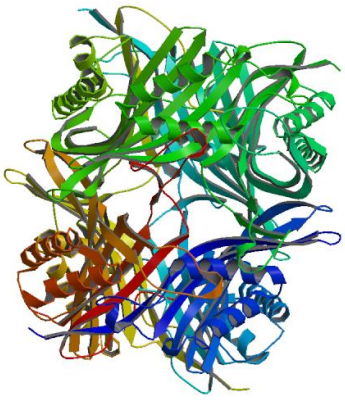

Figure 2: Template (6oe8.1.A) model

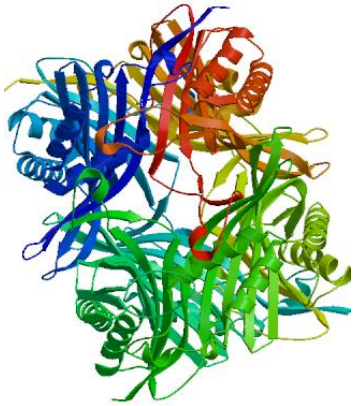

Figure 3: Uricase enzyme model

Verification and validation of Uricase enzyme model

The PDB format file of Uricase enzyme model was analyzed in Procheck and Verify 3D servers to determine the enzyme model quality. In Procheck server a Ramachardran Plot pertaining to Uricase model was obtained. In the Ramachandran Plot, organization of amino acids in different regions was displayed. In the Ramachandran Plot of Uricase enzyme model, 93.3%, 6.7%, 0.0% and 0.0% of amino acids were in the most favoured, additional, generously and disallowed regions, respectively (figure 4). Majority of amino acids in Ramachandran Plot (i.e., more than 90% of amino acids) were in most favoured regions inferring the good quality of Uricase enzyme model.

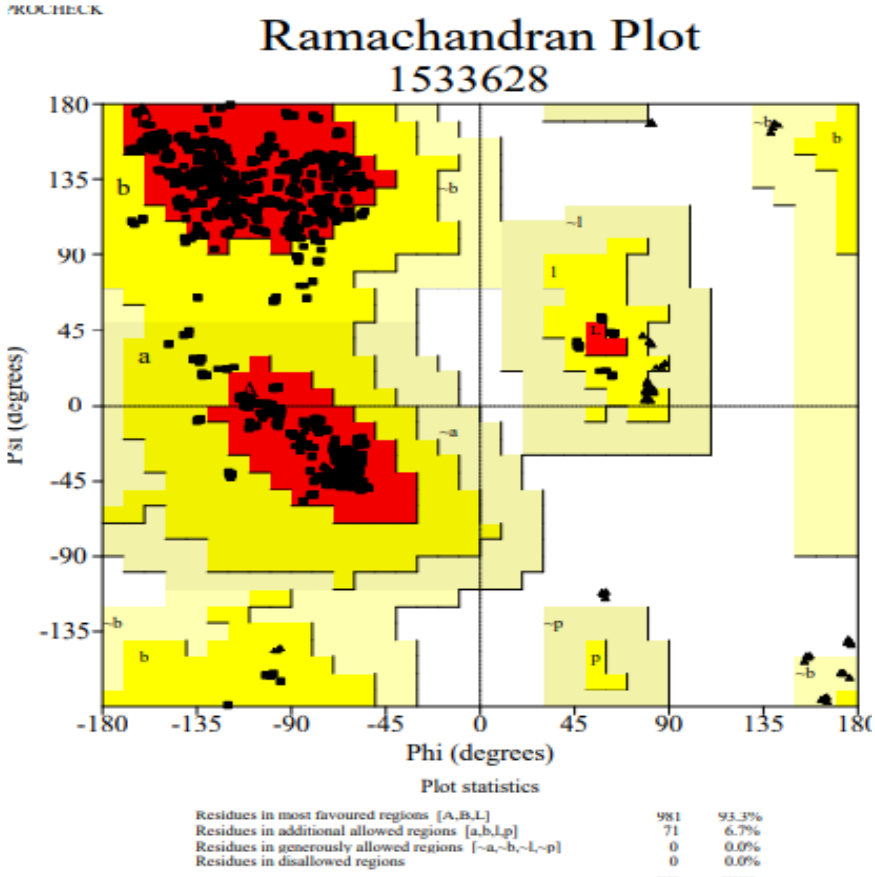

Figure 4: Ramachandran Plot of Uricase enzyme model generated in Procheck

In verify 3D, the 3D-ID score generated for the 83.82% of amino acids (figure 5) for Uricase enzyme model was >= 0.2. The model quality score of Uricase enzyme in verify 3D indicates its model reliability.

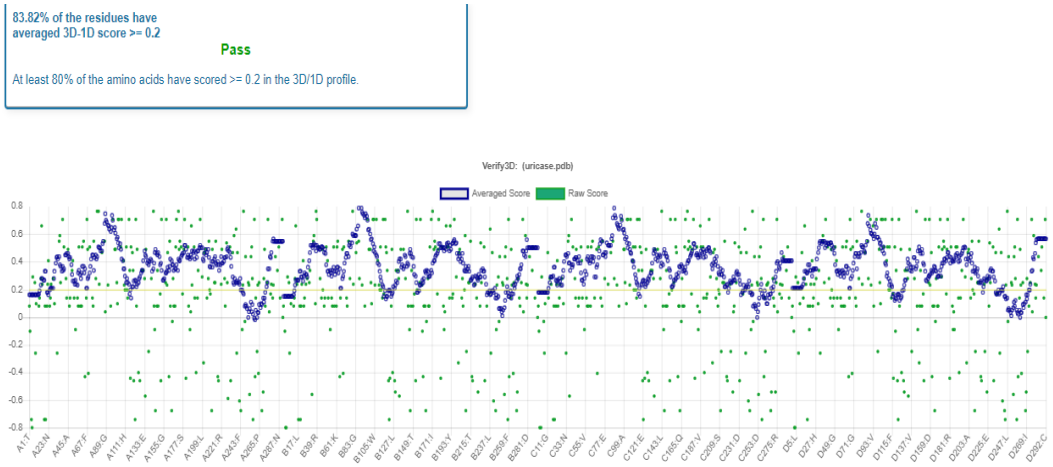

Figure 5: The 3D-ID score of Uricase enzyme model generated in Verify 3D

DISCUSSION

The FASTA format sequence of Uricase enzyme was derived from UniProt server. The UniProt server is a combination of various protein databases which includes TrEMBL, Ensembl, Swiss-Prot, PIR-PSD, EMBL, IPI etc. In UniProt various details like accession number and entry history of retrieved protein are displayed [9]. The Uricase sequence was analyzed in SWISS-MODEL to get corresponding Uricase model structure. In SWISS-MODEL the submitted protein sequence (query sequence) is aligned with the various sequences of proteins available in SWISS-MODEL to determine the protein sequence exhibiting highest similarity to query sequence [10]. The protein sequence of SWISS-MODEL showing maximum similarity is regarded as template. The template determined for Uricase enzyme was 6oe8.1.A. Based on template, 6oe8.1.A the model of Uricase was developed. The validity of Uricase enzyme model was checked in Procheck and Verify 3D. In Ramachandran plot of a protein model if more than 90% of amino acid residues are in most favoured regions indicates the good quality of the model. In verify 3D a model quality score is generated with respect to a submitted protein model. The model quality score is indicated as 3D-ID score. The 3D-ID score for a protein model is obtained based on the concurrence of various properties of amino acids in sequence to that of amino acids in three-dimensional model [11]. In Verify 3D the 3D-ID score of a protein model must be >= 0.2 for at least 80% of amino acids to validate its model. The 3D-ID score of Uricase enzyme model generated for 83.82% amino acids was >= 0.2 which determined the validity of Uricase model.

CONCLUSION

In the present work the web-based bioinformatics tools were extensively used to build and validate the model of Uricase enzyme which can be used as a potent drug against gout disease. From UniProt server the Uricase enzyme sequence was derived and submitted to SWISS-MODEL server to generate the Uricase three-dimensional structure. The quality of Uricase enzyme model structure was verified and validated using Procheck and Verify 3D servers. Further the Uricase enzyme can be improved by employing advanced bioinformatics tools. Its immunogenicity property can be minimized by replacing amino acids at specific sites by using sophisticated software tools. Even Uricase activity can be enhanced by employing In Silico methods. The improved Uricase enzyme can be produced by microbes by corresponding modification in Uricase gene in the laboratory.

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