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Research Article

3D Structure Modeling of Catalase Enzyme from *Aspergillus fumigatus*

Abstract

The respiratory diseases in humans, such as aspergilloma, allergic bronchopulmonary aspergillosis and invasive aspergillosis are caused by the fungal pathogen *Aspergillus fumigatus (A. fumigatus)* The enzyme catalase of *A. fumigatus* provides a putative virulence to this fungal pathogen against the toxic effects of human hydrogen peroxide, which they cleave into water and molecular oxygen. The 3-D structure of this protein in *A. fumigatus* is not known, while it is very important for understanding the molecular mechanism of action of this enzyme and development of new drugs for various respiratory diseases. This article proposes the 3-D structure of catalase enzyme from *A. fumigatus which has been predicted and validated using different computational programs.* Based on the percentage of residue occurrence in helical, strand and loop regions, four structural domains have been identified in the modeled structure. The structure and function relationship for all identified structural domains have been also described. This study will be helpful for *in silico* drug discovery against the virulence nature of *Aspergillus fumigatus.*

Introduction

In the last few years, several Aspergillus sp. have attracted much focus as opportunist pathogens as reflected in various published reports [1-4]. Fungi are able to cause diseases by overwhelming the host defense systems due to presence of several genes and proteins associated with their pathogenicity, called virulence factors [5]. The fungal pathogen Aspergillus fumigatus, member of Aspergillaceae family, has been associated with a wide spectrum of diseases in human, such as allergic bronchopulmonary aspergillosis, aspergilloma and invasive aspergillosis etc. [6]. It is one of the most ubiquitous fungi due to easy dispersal of its conidia [7]. It is calculated that a person could inhale several hundred conidia of A. fumigatus per day [7]. Alveolar macrophages and polymorphonuclear cells, cellular components of innate immunity of the lung, work against the conidia and hyphal form, respectively [8]. However, in vitro studies of neutrophil function have revealed that H_2O_2 effectively kills fungal hyphae [9] and that neutrophil-mediated damage is blocked by addition of a commercial catalase [10]. In A. fumigatus, three active catalases have been identified in which catalase enzyme present in the conidium is encoded by cat A gene [11], and two mycelial catalases are encoded by cat1/cat B [12] and cat2 genes [11]. It was reported that A. fumigatus conidial and mycelial catalases protect the fungus against hydrogen peroxide [11]. However, the inhibition of catalase activity could make A. fumigatus vulnerable to rapid killing by the H_2O_2 and accordingly, catalase which is a good scavenger of H_2O_2, was considered to be a putative virulence factor of A. fumigatus [13]. Hence, thorough study of 3D structure of the A. fumigatus catalase enzyme present in the above fungus can be helpful in understanding the mechanism of its defense against the host immune response and also in the prediction its molecular mechanism of infection.

Materials and Methods

Protein sequence data

In the present work, the primary full-length amino acid sequences of *A. fumigatus* catalase enzyme was searched and retrieved from GenPept, a protein sequence database of National Centre for Biotechnology Information (NCBI) *in fasta format.*

3-D structure Prediction, evaluation and validation

Template selection and 3-D structure of A. fumigatus catalase enzyme *was determined by HHpred server* by HMM-HMM comparison [14]. *The PDB file of the modeled structure was then downloaded after the completion of 3-D modeling. The model was visualized using PyMol [15]. The predicted 3-D model of A. fumigatus* catalase enzyme *was further validated using the programs; PROCHECK* [16], ProSA [17], ProQ [18] and Profile 3-D [19]. The PROCHECK was employed to check the valid stereochemistry and ProSA to detect the native structure compatibility. Also, ProQ, a neural network-based predictor based on a number of structural features was used to calculate the quality of the

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generated protein 3D model. The root mean square deviation (RMSD) of each atoms of the predicted model with respect to the template structure was also calculated using SuperPose tool [19]. Besides, the overall stereochemical quality of the protein, its amino acid residues in the allowed, disallowed region and overall G-factor were also evaluated by Ramachandran plot analysis [20].

Structural annotation and domain identification

The structural domains in the modeled structure of the catalase protein were recognized using DIAL server (http://caps.ncbs.res.in/DIAL/DIALserver.html) [21]. DIAL is an algorithm for domain identification in proteins where proteins are considered as a string of substructures (secondary structures and connecting loops) and the substructures are clustered according to their proximity indices. Each cluster, therefore, derived is a potential structural domain and disjoint factor is calculated for each domain organisation. This factor is a measure of compactness of the sub-structural clusters. Disjoint factor values more than 1.0 are considered to provide acceptable solutions of possible structural domain architecture for the protein of interest [21]. Based on the percentage of residue occurrence in helical, strand and loop regions, DIAL classifies the domains into four classes such as all alpha class, all beta class, alpha beta class and few secondary structure classes.

Structure and function relationship

The generated structure of the catalase protein was further analyzed using Sequence Annotated by Structure (SAS) server [22] and ProFunc server [16], for the structure and function relationship, and all identified domains, respectively.

Results and Discussion

3D structure prediction, evaluation and validation

The catalase (CAT) is one of the most active enzymes (EC1.11.1.6) present in archea, eubacteria, fungi, plants, and animals. It dismutates hydrogen peroxide (H_2O_2) into one dioxygen (O_2) and two water molecules [23]. Besides monophyletic monofunctional catalases, other enzymes, such as catalase-peroxidases, Mn-catalases, peroxidases and peroxiredoxins contribute to H_2O_2 disposal. H_2O_2 is inevitably formed in cells: most of it comes from superoxide (O_2^-) dismutation and from the activity of some oxidases [24,25]. Hence, a complete sequence of 749 amino acid residue for the A. fumigatus catalase enzyme was searched at the GenPept database and saved in fasta format (Accession no. AAB47761.1*) It was reported that all the catalases share a highly conserved core structure but have nevertheless functional differences [26, 27]. Therefore, the homologous proteins with known structure were explored for the template selection of target protein in PDB database updated on 16 November 2013 using HHpred server. It was observed that the* crystal structure of the catalase-1 from *Neurospora crassa* (PDB id: 1sy7_A) showed maximum similarity with the *A. fumigates catalase selected* as the template with the sequence similarity of 68% and E-value 5.1e-177. The predicted 3-D structure satisfied all the validation criteria on the basis of PROCHECK are illustrated in the Figure 1. According to the Ramachandran plot analysis of the predicted structure, 91.4% residues of Φ/Ψ angles are in the most favoured regions, 7.5% residues in the additional allowed region, 0.2% residues in generously allowed region and 0.9% residues in disallowed region as shown in the Figure 2. Additionally, the overall value of -0.08 was observed for G-factor, suggesting that the structure is highly unusual. Also, the ProSA server revealed that the model structure of the *A. fumigatus catalase enzyme* occupied the same region as observed in the X-ray predicted native protein structures with Z-score of -9.24 (Figure 3) It was also observed that the overall residue energies of the *A. fumigatus* catalase model were largely negative except for some peaks in few regions.

Besides, the results from the ProQ shows that the predicted structure is more reliable with LG score 3.77 and MaxSub 0.348. The overall quality for final structure has been further evaluated by Verify3D. The compatibility scores and the result for the

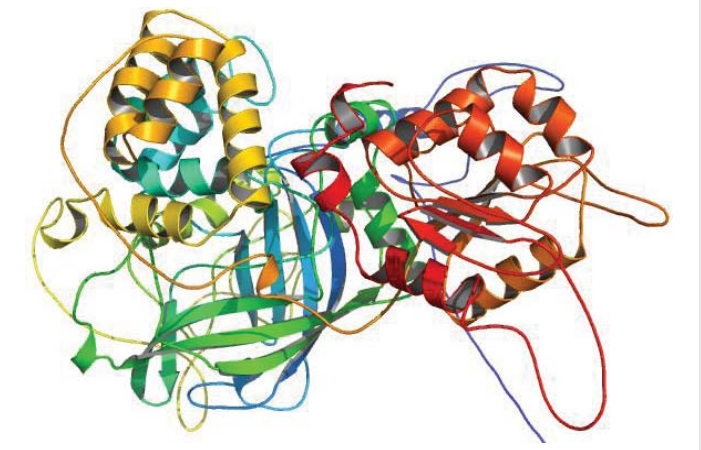

Figure 1: Predicted 3D structure of *A. fumigatus* catalase.

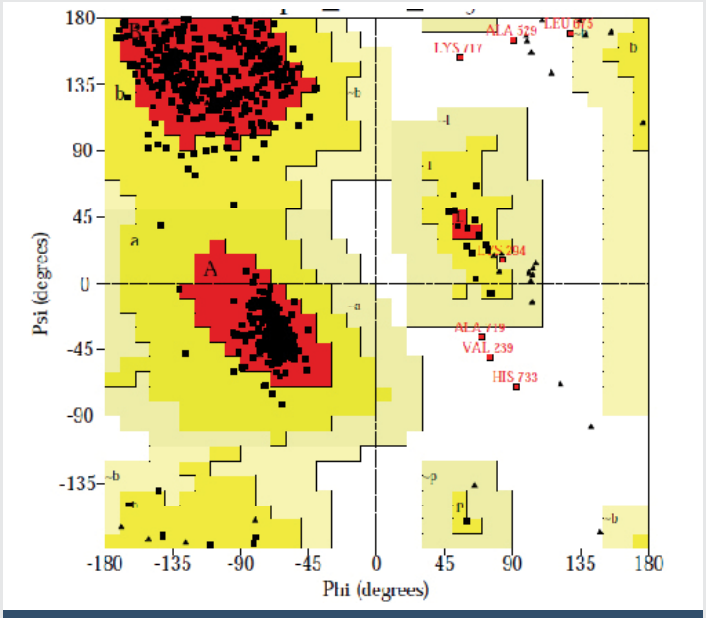

Figure 2: Ramachandran plot of predicted structure.

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final structure are presented in the Figure 4. The compatibility scores for all the residues in the developed model are above zero and hence, inferred that the generated 3D model for the catalase is reliable. The RMSD between predicted structure and template structure was found as 2.05A°, suggesting that the predicted 3D structure is an accurate model for the *A. fumigatus catalase enzyme*. The validated model was deposited in Protein Modeling Database with PMID PM0079726. Besides, with these evaluations, 3D model of *A. fumigatus* catalase was valid enough for high throughput virtual screening (VHTS) for designing potential antifungal drug.

Structural annotation and domain identification

The SAS server was used to generate the wiring diagram as depicted in Figure 5. The ProMotif documentation of the enzyme via Profuncserver showed the results for the secondary structure summary as: the 749 residue span of the structure consists of 112 residues (15%), which were involved in the formation of the strands, 189 residues (25.2%) for the alpha helices, 26 residues (3.5%) for the 3–10 helix and 422 residues (56.3%) for the several other structural moieties. Also, the results show 4 beta sheets, 3 beta-alpha-beta motifs, 9 beta hairpins, 2 psi loops, 4 beta bulges, 21 strands, 30 helices, 29 helix-helix interactions, 64 beta turns and 9 gamma turns.

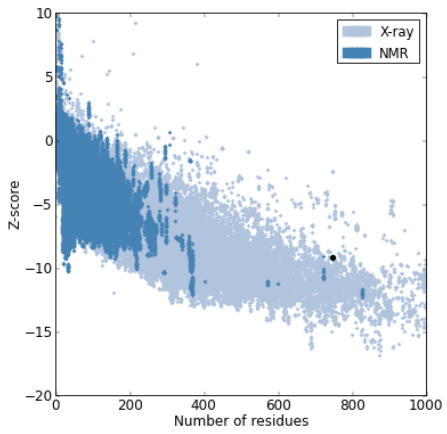

Figure 3: Validation of predicted enzyme using ProSA

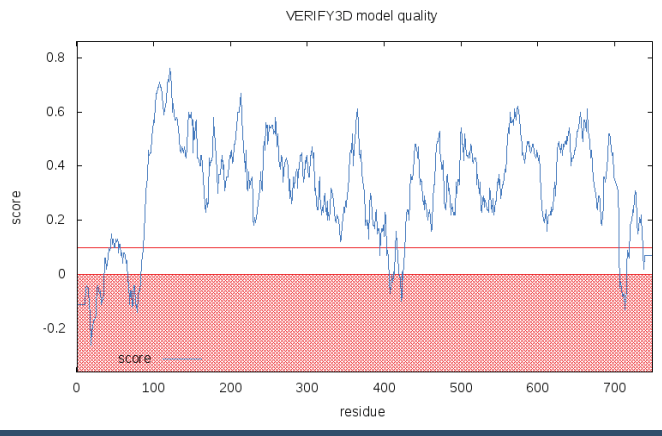

Figure 4: Verify3D graph of 3D model of protein having highest 3D-1D averaged score (0.76).

Additionally, the four structural domains were also identified in the predicted 3D structure. The first domain related to few secondary structures class comprising 18 residues were detected from Met1 to Asn18, the second domain belonging to Alpha Beta class involves 497 residues was observed from Thr19 to His37 and Glu85 to Arg562, the third domain related to few secondary structures class consisting of 47 residues were established from Thr38 to His84 and the fourth domain belonging to Alpha Beta class consisting of 187 residues were found from Ala563 to Phe749. The 3-D structures of these domains are depicted in the Figures 6-9, respectively.

Structure and function relationship

Protein structure-function relationships can be investigated by asking how nature has reengineered protein structures to perform a variety of functions. Computational methods directed at the identification and analysis of related protein structures are an important prerequisite in this endeavour [28,29]. The structural domain regions were highlighted in predicted structure (Figure 10) SAS server reported three

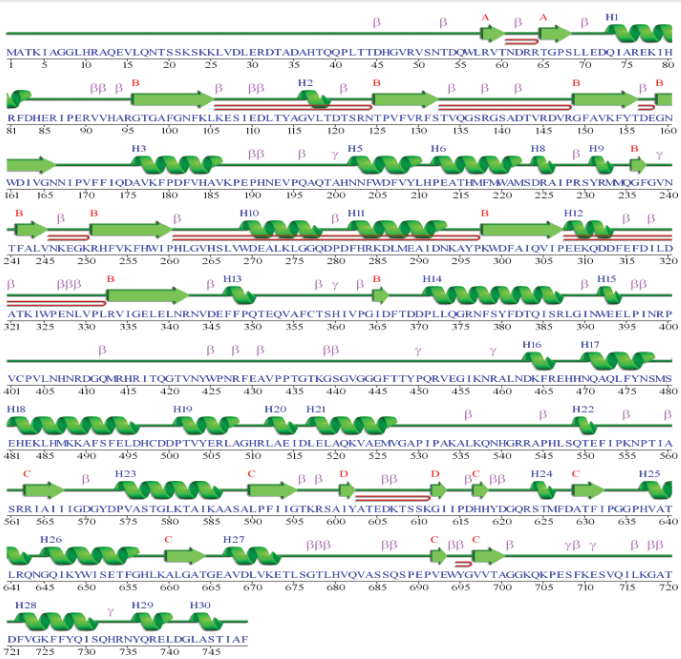

Figure 5: Wiring diagram of the predicted structure *A. fumigatus* catalase.

Figure 6: Structure of first structural domain.

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catalytic residues His93, Ser132 and Asn166 in the predicted structure while the positions of these catalytic residues are completely different in the template structure (His92, Ser131 and Asn165) [30]. This suggests that there is an insertion type mutation in the *Aspergillus fumigatus* catalase when compared with *Neurospora crassa* catalase-1. The catalytic residues of the predicted structure were observed to be the part of second structural domain. The residues His 93 and Asn166 were found to form contact with hydrogen peroxide and many ligands, while Ser132 was found to contact with protoporphyrin IX containing Fe. Lys274 and Ala460, the part of second structural domain and Ser685, Glu693, Trp694 and Val697, the part of fourth structural domain were found to contact calcium ion. His84 found to contact chloride ion which is the part of third structural domain.

Conclusion

The present study projected the 3D structure of *Aspergillus fumigatus* catalase. The structure to function relationship was also established by identifying the functional role of important residues in the four different structural domains of the modeled structure. This study purposed to discover new ligand with therapeutic potential to treat the virulence nature of this fungus for controlling the respiratory diseases.

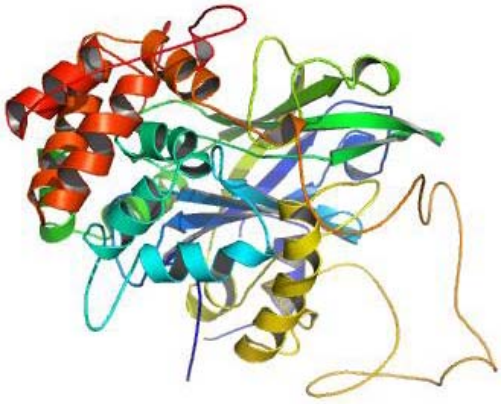

Figure 7: Structure of second structural domain.

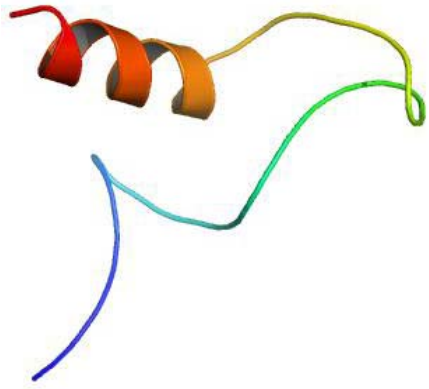

Figure 8: Structure of third structural domain.

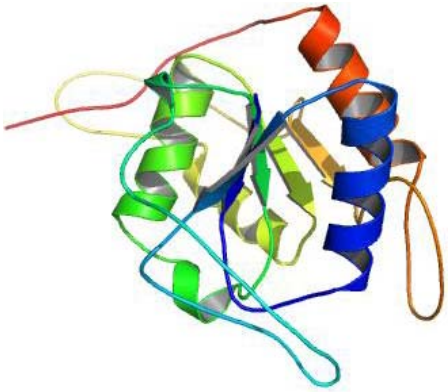

Figure 9: Structure of fourth structural domain.

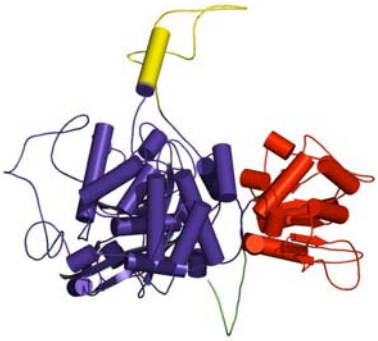

Figure 10: Representation of all structural domains in the predicted structure: Green colour represents the first domain, purple colour depicts the second domain, yellow colour shows third domain and red colour displays fourth domain. All the helices are shown in cylindrical form.

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