Vol. 3, No. 2: 100-102 (2015).
ISSN: 2313-3937

WORLD JOURNAL OF EXPERIMENTAL BIOSCIENCES

Research article

Three dimensional structure of AFLR2 produced from *Aspergillus parasiticus*

Nemat J. Al- judy[1]*

ABSTRACT

Present study focused on the structure of Aflatoxin B1 Regulatory Gene in that isolated from environmental *Aspergillus parasiticus* isolate. After purification of the studied Aflatoxins are polyketide derived secondary metabolites produced by A. parasiticus spp. The toxic effects of aflatoxins are adverse consequences on human health and agricultural economics. The aflR2 gene is regulatory gene for aflatoxin biosynthesis encodes a protein containing a zinc-finger DNA-binding motif. AFLR-Protein three-dimensional model was generated using Robetta server. The modeled AFLR-Protein was further optimization and validation using Rampage. In the simulations, we monitored the backbone atoms and the C-α-helix of the modeled protein.

Keywords: aflR2 gene, Aspergillus parasiticus, C-α-helix.

Citation: Al- judy NJ. (2015) Three dimensional structure of AFLR2 produced from *Aspergillus parasiticus*. *World J Exp Biosci* **3:** 100-102.

Received September 7, 2015; Accepted October 5, 2015; Published October 22, 2015.

INTRODUCTION

Aflatoxins are a group of mycotoxins with potent toxicity and carcinogenicity toward mammals. They are produced by some strains of *Aspergillus parasiticus* and they can be found as contaminants in a wide variety of food and feed commodities. Aflatoxin contamination in agricultural products is a serious problem from the viewpoint of food safety, and also that of economical loss [1, 2]. However, it is difficult to resolve the problem because of the lack of an effective method to control aflatoxin production. Recent advances in cloning several aflatoxin pathway genes revealed that most of these genes are clustered within a 60-kb DNA region in *A. parasiticus* and *A. flavus*. [3,4]. This finding has spawned renewed interest in the study of the regulation of aflatoxin biosynthesis. Previous studies have suggested that one of these genes, *aflR*, is involved in some aspect of the regulation of aflatoxin biosynthesis. In the present study, 3D-Structure of AFLR-Protein was performed using Robetta Server. The constructed model was further validated by Ramachandran plot. The refined mo-

*Correspondence: Al- judy NJ.neemaaljudy@yahoo.com.
Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq.
Full list of author information is available at the end of the article

del was again subjected to energy minimization using GROMOS and evaluated for quality assessment. In present study the model of AFLR2 produced from *A. parasiticus* was established.

MATERIALS AND METHODS

Sequence alignment and Protein structure

3D-Structure of AFLR-Protein was performed using Robetta server. The amino acid sequence of AFLR-Protein was retrieved from NCBI. The Robetta server is an automated protein structure prediction service for *ab initio* and comparative modeling [5]. Initially server searches for structural homologs using BLAST or PSI-BLAST [6] and then it breaks down the target sequence into its individual domains, or independently folding units of proteins, by matching the sequence to structural families in the Pfam database. Domains with structural homologs then follow a template-based model (i.e., homology modeling) protocol. The final structure prediction is selected by taking the lowest energy model as determined by a low-resolution Rosetta energy function. The backbone conformation of the modeled structure was calculated by analyzing the phi (F) and psi (ψ) torsion angles using Rampage [7].

The GROMACS 4.5.4 package [8] was used to run molecular dynamics simulations with the GROMOS96 43a1 force field and the SPC216 water model. The modeled AFLR-Protein was energy minimized to discard the high-energy intramolecular interactions. The overall geometry and atomic charges were optimized to avoid steric clashes. Then the whole system was gradually heated from 0 to 300 K over 500 ps using the NVT ensemble run with the Berendsen procedure and, subsequently in 500 ps NPT ensemble run at pressure of 1atm. The Parrinello- Rahman pressure coupling has been used. Finally, a 10 MD simulation was carried out to examine the changes and dynamic behaviour of the protein was analyzed by calculating the RMSD and energy.

Protein functional site

Q-Site Finder [9] server was used to predict the structure based protein functional site.

RESULT AND DISCUSSION

Robetta server is an automated protein structure prediction service for *ab initio* and comparative modeling [5]. The server searched for structural homolog using PSI-BLAST, and then it breaks down the target sequence into three individual domains. The domains with structural homolog follow a template model (3 coqA). The final structure prediction was done using 3coqA as template model (**Fig 1**). Geometric evaluations of the modeled 3D structure were performed using Rampage [7] by calculating the Ramachandran plot (**Fig 2**). The plot (**Fig 2**) of our model shows that 98.2% of residues were found in the favored and 1.4% allowed regions and 0.5% were in the outlier region.

Fig 1. Ramachandran plot for the AFLR2-Protein model.

Fig 2. Modeled structure of the AFLR2-Protein.

Simulations of AFLR-Protein

Molecular dynamics aimed to explain protein structure and function problems such as structural stability, folding and conformational flexibility. In the simulations, we monitored the backbone atoms and the C-α-helix of the

modeled protein. The RMSD values of the modeled structure's backbone atoms were plotted as a time-dependent function of the MD simulation. The results support our modeled structure, as they show constant RMSD deviation throughout the whole simulation process. The time dependence of the RMSD of the backbone atoms of the modeled protein during a 10 ns simulation is shown in **fig 2**. The graph clearly indicates that there is a change in the RMSD from 2.0 Å to 4.0 Å in the AFLR-protein during the first 1000 ps, but after that it reaches a plateau. The RMSD values of the backbone atoms in the system tend to converge after 2000 ps, showing fluctuations of around 1 Å. The low RMSD and the simulation time indicate that, as expected, the 3D structural model of AFLR-protein represents a stable folding conformation.

Identification of functional site

Q-Site Finder server was used for the prediction of functional sites in the modeled AFLR-protein. These servers detected the following putative functional site residues in the modeled protein: LEU-255, THR-270, VAL-273, ILE-274, ASN-277, LYS- 278, THR-281, LEU-307, LEU-310, ALA-311, TYR-313, ALA-314, ALA-317, THR-319, GLN-320, CYS-321, THR-322, SER-323, and THR-324 with significant matches.

Conclusion

The model generated by using the template 3coqA proved to be the best model generated as compared to the other templates. The modeled AFLR-Protein was further optimization and validation using Rampage by calculating the Ramachandran plot.

Conflict of interest

The author declares that she has no conflict of interests.

REFERENCES

1. **Konietzny U, Greiner R.** (2003) The application of PCR in detection of mycotoxigenic fungi in food. *Braz J Microbiol* **34**: 283-300.
2. **Livak K, Schmittgen T.** (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta DeltaC(T)) **method**. *Methods* **25**:402-408.
3. **Manonmani HK, Anand S, Chandrashekar A, Rati ER**. (2005) Detection of aflatoxigenic fungi in selected food commodities by PCR . *Process Biochem* **40**: 2859–2864.
4. **Passone MA, Rosso LC, Ciancio A, Etcheverry M.** (2010) Detection and quantification of Aspergillussection **Flavispp**. In stored peanuts by real time PCR of nor-1 gene, and effects of storage conditions on aflatoxin production. *Int J Food Microbiol* **138**: 276-281.
5. **Peplow AW, Tag AG, Garifullina GF, Beremand MN.** (2003) Identification of new genes positively regulated by *Tri10* and a regulatory network for trichothecene mycotoxin production. *App Environ Microbiol* **69**: 2731-2736.
6. **Price MS, Conners SB, Tachdjian S, Kelly RM, Payne GA.** (2005) Aflatoxin conducive and non-conducive growth conditions reveal new gene associations with aflatoxin production. *Fungal Genet Biol* **42**: 506-518.
7. **Rossen L, Nosrskov P, Holmnstrom K, Rasmusspm OF.** (1992) Inhibition of PCR by Components of food samples, microbial diagnostic assay & DNA extraction solusions. *Int J Food microbial* **17**: 37-45.
8. **Schmidt-Heydt M, Abdel-Hadi A, Magan N, Geisen R.** (2009) Complex regulation of the aflatoxin biosynthesis gene cluster of *Aspergillusparasiticus*in relation to various combinations of water activity and temperature. *Int J Food Microbiol* **135**: 231-237.
9. **Schmidt-Heydt M, Rufer CE, Abdel-Hadi A, Magan N, Geisen R.** (2010) The production of aflatoxin B1 or G1 by *Aspergillus parasiticusat* various combinations of temperature and water activity is related to the ratio of aflS to aflR expression. *Mycotoxin Res* **26**: 241-246.

Author affiliation:

1. *Department of Biology, Collage of Science, University of Baghdad, Baghdad, Iraq.*