International Research Journal of Advanced Engineering and Science
ISSN (Online): 2455-9024

Genomic Sequence Analysis of *Aspergillus fumigatus* Lipoxygenase: A Causative Agent of Severe Asthma

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***Abstract*—**
Background: *Bronchial asthma is an inflammatory disease of the airways which may be worsened due to numerous extrinsic factors. The most common trigger is continuous exposure to allergens of which fungal agents are important factors. There is overwhelming evidence for the presence of fungal sensitization in patients with asthma. It has been proposed that fungal lipoxygenase enzymes and their eicosanoid products are crucial in asthmatic diseases. Human 5-lipoxygenase derived leukotrienes induce inflammation, mucus secretion, vasodilation, and bronchial constriction. Research has also shown that the fungal pathogen Aspergillus fumigatus is capable of secreting LoxB which is a 5-lipoxygenase homolog and it participates in eicosanoid production, including leukotrienes. LoxB is translocated into the lung epithelial cells where it participates in the production of leukotrienes and other eicosanoids, and induces asthmatic responses, such as bronchoconstriction. Analysis from this study gives an idea of facts needed in the structure-based drug design geared towards tackling this disease and this design relies solely on the knowledge of the three-dimensional structure of the biomolecular target which is the lipoxygenase enzyme.*
Results: *In this report, we present a computational analysis of the nucleotide and amino acid composition of lipoxygenase obtained from Aspergillus fumigatus. This in turn was used in the protein secondary and 3D homology modelling necessary for a structure based drug design. The enzyme sensitivity to antifungal drugs was also predicted using results obtained from the protein disordered region analysis.*
Conclusions: *Antifungal drugs designed specifically to target the fungal lipoxygenase tend to act fast based on the enzyme instability. These drugs take advantage of the cytosolic instability of the disulfide bonds which has been analysed also to be of a very minute quantity in the enzyme.*

***Keywords*—** *Lipoxygenase, Eicosanoid, Leukotrienes, Vasodialation, Bronchoconstriction.*

I. BACKGROUND

Fungi have long been associated with asthmatic diseases, yet the exact mechanism(s) by which fungi induce asthma is unknown [31]. Fungal exposure is inevitable in human existence, and this frequently leads in disease [28]. Allergy to fungi causes asthma severity in very large numbers of people affected by asthma [17]. Fungi cause problems to the lung in two ways; either by acting as aeroallergens or as a pathogen causing infection [9]. Some fungi can do both, often simultaneously [23]. Fungi that causes lung infection must be able grow at body temperature and this property is restricted to a relatively narrow range of fungi, mostly yeasts and members of the *Aspergillus* genera [26]. The most common fungus that causes lung infection is the *Aspergillus fumigatus*, although other *Aspergillus* species also can [20]. While most asthmatic patients have mild symptoms, which are well controlled with anti-inflammatory and bronchodilator therapy, a very few have a severe airway inflammation and airflow obstruction that requires multiple hospital admissions [25]. The reasons for these differences in the severity of asthma in different patients are complex and are yet not fully understood [33]. There are many different phenotypes of "severe" asthma, including brittle asthma, and the differentiation of these more severe phenotypes is somewhat subjective [1], [2]. Even the definition of asthma severity is complex [13]. It is usually based on the expression of symptoms, although it is clear that many of the symptoms expressed in this group are not caused by either airway inflammation or bronchospasm [27]. Many authors use the term "severe" to represent those with symptoms that are definitely related to asthma, and the term "difficult" is reserved for those who use healthcare resources with symptoms that are only indirectly associated with asthma [12], such as vocal card dysfunction and dysfunctional breathing [32]. An *Aspergillus fumigatus* strain that overexpresses the human 5-lipoxygenase homolog "LoxB" was developed to fully understand its impact and this increased the production of eicosanoids known to lead to airway hyperresponsiveness and increased mucus production [29].

II. MATERIALS AND METHODS

Sequence Mining

Data on the amino acid sequences of the *Aspergillus* lipoxygenase was obtained from the GenBank database. The GenBank sequence database is an open access, annotated collection of all publicly available nucleotide sequences and their protein translations. This database is produced and maintained by the National Center for Biotechnology Information (NCBI) [22].

Sequence Composition

The total nucleotide and amino acid composition of lipoxygenase in *Aspergillus fumigatus* was determined and analyzed using the MEGA 7 software [24].

Secondary Structure Prediction

The Aspergillus Lipoxygenase secondary structure was generated using the Chou and Fasman secondary structure prediction server (CFSSP) [21].

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International Research Journal of Advanced Engineering and Science
ISSN (Online): 2455-9024

3D Structure Generation

The 3D structure was generated using the Swiss Model Homology Software. Target search with BLAST was performed against the SWISS-MODEL template library [15, 30].

Protein Disordered Region Prediction

The DisEMBL software was used in predicting the protein disordered region. This uses three different criteria (Coils, Hot loops and Remarks/Missing coordinates) for defining which residues are disordered [6]. However, protein disorder is only found within loops. The graph could not be displayed because of its complexity [8].

III. Results

Amino acid sequence of lipoxygenase from Aspergillus fumigatus Af293

>gi|70982632|ref|XP_746844.1| lipoxygenase [*Aspergillus fumigatus* Af293]
MPQNTIALTPGVVLGHPEVQEKWPRNPEDLAVSDIDTGVLI
NELSNINLLPGKVRVLEENPEIMQAKTYE
KPPPIEEGTYRGTQLALTKIYNLVEQRFSSFMDVANFEPLV
PSPLTKDQKRKFFAFTDGSDGYPPHLNLA
GNVEAAKKDESNQRSTLQPSQIFDKMRLLQLASLLPSIVPN
KFIRELARLTARGWFGSMGRPDAGEKLKD
VEDYNRRARGWYFGKDIFDLPNVGDLPDWYSDARFAQQHFT
GTNPTTIEQASDEWSEHFITAAEAPEDGP
VKEILTDLRNSCRKSLYMQDYSYFRKAAGLDPTAVIKCEFD
EAGKKRHRYGCASVCLFYLNDQGQLYPLA
IVIDWRGKAETSVTIYNRELIKRKDLRAGGEKQDQKHKVTD
EAHDWAWRYAKTCVQCSDWLRHEVTVHLT
NTHMIEEAIIVASHRQLDPDHPVMLLLYPHWQKTLALNAAA
RNTLVPHVIVDLIGFQASEGFAFIRHAYE
NFDFKGRYVPTDLRQRGFPPEELDSPKFHNYAYARCINSMW
HKIRSYVQDMLALAYPGTDADHKVRNDQC
IQAWSDEMRSSDGARLPSFPTISTFEELVDCVTMCIHIASP
QHTAVNYLQNYYQSFVVNKPPCLYTEPPT
SLQSLLGYTEKELVEALPMNHPREWLLASHIPYLLSFKPGN
KESLIVYAASKFRVYHNKPTEKDQAIAAA
TGKFNTALAESQEEFKRYGQATDDWETVEYEVLSPEWNAVS
ILI

The sequence above is the amino acid sequence of lipoxygenase from the Af293 strain of Aspergillus used for the analysis in this study. The sequence was obtained from the NCBI Genbank using the BLAST search tool which produced results in the Fasta format.

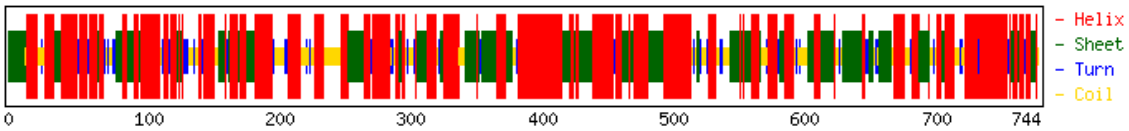

Fig. 1. Predicted secondary structure of lipoxygenase from the Af293 strain of Aspergillus fumigatus

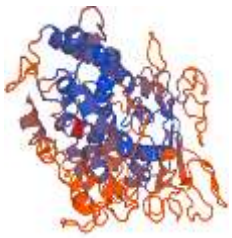

Fig. 2. The 3D protein homology structure of lipoxygenase from the Af293 strain of *Aspergillus fumigatus*

Regions of the α-helices, β-sheets, turns and coils are shown in red, green, blue and yellow respectively. The result from this shows residues of 518, 291, 103 for the helix, sheets and turns respectively. This respectively gives a percentage of 69.6, 39.1 and 13.8 for each.

The 3D structure was obtained using a structural bioinformatics web server dedicated to homology modeling of protein three-dimensional structures "SWISS-MODEL". Homology modeling amongst other methods was chosen because it is currently the most accurate method to generate reliable three-dimensional protein structure models and is routinely used in many practical applications.

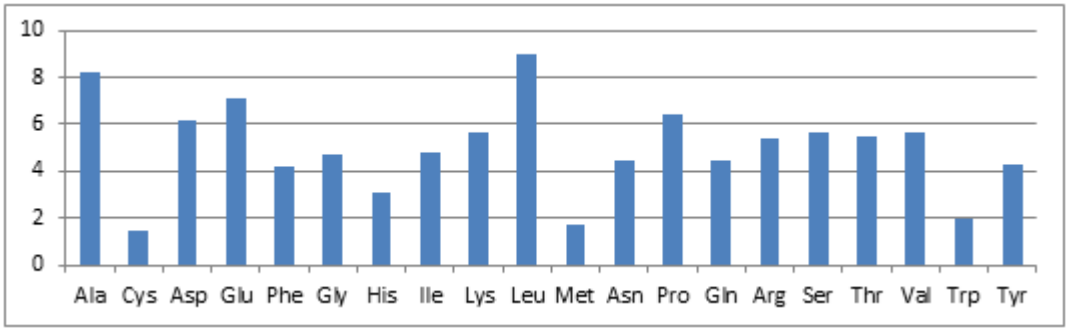

Fig. 3. The percentage amino acid composition of lipoxygenase from the Af293 strain of *Aspergillus fumigatus*

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International Research Journal of Advanced Engineering and Science
ISSN (Online): 2455-9024

The amino acid composition graph showed on the X-axis each amino acid. The percentage composition of each amino acid in lipoxygenase is shown on the Y-axis. This was constructed using the Mega 7 software which made use of the information available from the downloaded Fasta format of the amino acid sequences.

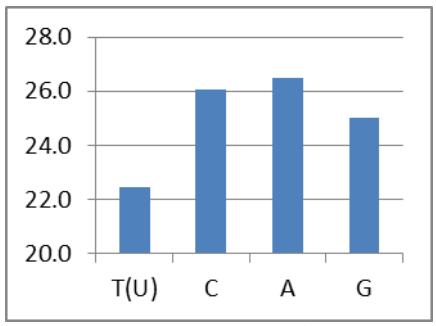

Fig. 4. The percentage nucleotide composition of lipoxygenase obtained from the Af293 strain of *Aspergillus fumigatus*

The nucleotide composition graph show on the X-axis each Nucleotide (T, C, A, G) and the percentage composition on the Y-axis. This was also constructed using the Mega 7 software which made use of the information available from the downloaded Fasta format of the Nucleotide sequence.

The disordered regions of the analysed amino acid sequence of lipoxygenase obtained from the Af293 strain of *Aspergillus fumigatus* was viewed using the DisEMBL software. This distinctly shows the flexible linkers i.e the Loops/Coils in calculating the disorder probability.

IV. Discussion

The BLAST search result show the amino acid sequence of lipoxygenase for the *Aspergillus fumigatus* strain of interest in the FASTA format [3]. This was extracted for the purpose of this study.

Information about the amino acid composition of a given protein is important in the determination of its structure, function and stability [4]. The result of the amino acid composition showed that lipoxygenase from *Aspergillus fumigatus* is highly composed of alanine and leucine. alanine and leucine are both amino acids with non-polar side chains; this indicates that the *Aspergillus fumigatus* lipoxygenase is a cytosolic enzyme which folds in a polar/aqueous environment due to the hydrophobic interaction between the non-polar side chains of its amino acids [5].

Generally, information about the composition of nucleotides in a given gene is very important in the aspect of primer design for gene amplification in the Polymerase Chain Reaction which takes into consideration the percentage of the guanine-cytosine in the total nucleotide composition of the gene [7]. The result of the nucleotide composition showed that lipoxygenase from *Aspergillus fumigatus* have a high concentration of guanine and cytosine. This high concentration of guanine and cytosine invariably translates into an increase in the melting temperature "Tm" required for the denaturation of the lipoxygenase gene which will serve as the template DNA in the polymerase chain reaction [14].

Proteins containing linear peptide motifs (Disordered regions) are enriched to be dosage sensitive [19]. Results from the analysis of the protein disordered regions of lipoxygenase in *Aspergillus fumigatus* as revealed by the DisEMBL server graph has shown that lipoxygenase from *Aspergillus fumigatus* contains numerous disordered regions and as such tend to be more drug sensitive [10].

Observations from the amino acid composition graph have shown that the lipoxygenase enzyme from *Aspergillus fumigatus* exhibits a high level of stability due to their high concentration of cysteine [11]. Structurally, cysteine belongs to the sulfur amino acids because of the sulfur atom appearing in its side chain. The sulfur atom of cysteine is involved in the formation of sulfhydryl group which is very reactive [16]. The oxidation of two cysteine residues forms a dimer containing disulfide bridges which is most important for the stability of the enzyme tertiary and quaternary structure [18]

V. Conclusion

The sequence analysis of lipoxygenase from the Af293 strain of *Aspergillus fumigatus* has shown to a great extent the various important properties of the enzyme ranging from its primary, secondary and tertiary structure. With the realization of the prevalence of protein disordered regions and the important roles they play in a cell as potential drug targets, the knowledge of the *Aspergillus fumigatus* lipoxygenase disordered regions will be a necessary tool in the growing pharmacological drug design and development approach targeted at curbing the enzymatic action of the protein as the causative agent of the disease "Asthma" since the biochemical procedure to which it achieves this is still yet unclear.

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