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In-silico Homology Modeling of MMP25 involved in Asthma

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ABSTRACT

Asthma is a chronic long term inflammatory disease of the airways of lungs in which the airways narrow, swell and produce extra mucus. This leads to a limitation of the flow of air to and from the lungs, causing shortness of breathing. According to the latest WHO estimates released in December 2016, there were 3,97,000 deaths in 2015 and about 334 million patients are suffering from asthma across the world. It is caused by a combination of genetic and environment factors. Exposure to second hand smoke, exhaust fumes or other types of allergens is the major risk factors for developing asthma. Subsequent work suggest that the MMP25 protein plays a crucial role for causing airway remodeling in asthma disease by cleaving fibronectin, a extracellular component secreted by fibroblast which maintains the form, solidity and integrity of the lungs. In the present study, we used different In-Silico tools and techniques which includes retrieval of MMP25 protein sequence from UniProt KB database and the sequence analysis was performed by using Protparam tool which concluded that the protein was unstable and basic in nature. The secondary structure was predicted by using GOR tool which indicated that the percentage of coils was higher than the percentage of alpha helix and extended strand. Then the 3D structure of MMP25 was predicted by using SWISS MODEL server and the model was validated by using PROCHECK analysis tool. After validation of the model, the validation score was 89.40% indicating that the model was of good quality and the predicted 3D structure was deposited in protein model database (PMDB). Further study on MMP25 protein can be carried out in molecular docking and in structure based drug designing (SBDD) to inhibit airway remodeling in asthma.

Keywords: In-Silico, Asthma, MMP25, UniProtKB, Homology Modeling

I. INTRODUCTION

Asthma (Greek word, asthma meaning 'panting') is a common chronic long term inflammatory disease of the airways of lungs in which the airways narrow, swell and produce extra mucus [1]. It is characterized by recurrent symptoms, reverse obstruction of the airways, airway inflammation and bronchial hyper responsiveness [2]. It was first recognized in ancient Egypt and then it was officially named as a respiratory problem by Hippocrates circa 450 BC [3]. In 12th century, the Jewish physician - philosopher Maimonides wrote a treatise on asthma in Arabic, in which he described about the symptoms and means of treatment [4]. Asthma is caused by a combination of genetic or environmental factors [5]. If a person is suffering from asthma before age 12, then it is due to genetic factor and after age 12 it is due to environmental factors [6]. Genetic factors that causes asthma may be due to family history with different genes involved [7]. Environmental factors mainly include outdoor allergens such as pollens from grass and weeds, indoor allergens such as dust mites, pet dander and mold, irritants in the air such as smoke,

chemical fumes and strong odors, cold air or dry, wet or windy weather, low air quality due to traffic pollution or high ozone levels and exposure to volatile organic compounds. Asthma cannot be cured, but its symptoms can be controlled [1]. Symptoms mainly include coughing, shortness of breathing, chest tightness, wheezing and this symptoms may vary from person to person [8]. The diagnosis of asthma requires lung function tests. Common lung function tests are spirometry, peak airflow and FeNotest (exhaled nitric oxide) [9].Important management strategies are reduction of exposure to allergens, testing the severity of symptoms, avoiding the triggers, regular usage of medications. There are about 334 million patients suffering from asthma across the world [10]. It has been estimated that by the year 2025, additional 100 million peoples are excepted to develop asthma worldwide [10]. INSEARCH, GINA AND WHO survey suggests that the prevalence of asthma in India varies between 2.05 to 3.5% (17-30 million patients) [11]. The estimated cost for asthma treatment per year has been calculated to be approximately 139.45 billion [12]. According to the latest WHO estimates released in December 2016, there were 3,97,100 deaths due to asthma in 2015 [13]. Matrix metalloproteinases (MMP's) are calcium dependent zinc containing endopeptidases [14] and were first identified in vertebrates in the year 1962 including humans [15]. MMP's mainly function at neutral pH [16] and they are secreted in their latent form as proenzymes or pro-MMP's [17]. They play a major role in embryogenesis and in normal physiological conditions, such as proliferation, cell motility, remodeling, wound healing, angiogenesis [18,19].They were first described by Jerome Gross and Charles Lapiere (1962) [20]. MMP's have a multidomain structure that consist of a prodomain, a catalytic domain, a hinge region and a hemopexin domain [21]. Total 26 different MMP's and 24 of their coding genes were identified [16,21]. On the basis of substrate specificity, sequence similarity, and domain organization, vertebrate MMPs are divided into seven major groups: collagenases, gelatinases, stromelysines, matrilysins, Enamelysin, Metalloelastase, membrane type MMPs, and other MMP's [17,21,22]. Matrix metalloproteinase-25 is a membrane type protein present in humans and it is encoded by the MMP25gene [23,24,25]. It is also a GPI-anchored proteinase which is expressed in leukocytes hence named as leukolysin [26,27]. It is also expressed by eosinophils, neutrophils, and mast cells [28]. Leukolysin degrades extracellular matrix components and promotes airway bronchial remodelling [28].It also cleaves collagen type 4, gelatin, fibronectin and fibrin. Fibronectin is a extracellular component secreted by fibroblast which maintains the form, solidity and integrity of the lungs. As the fibronectin gets cleaved by MMP25, it promotes airway remodeling in asthma due to the altered fibroblast.Thus, MMP25 plays a key role at various levels of the inflammatory process. In the present study, we used different *In-Silico* tools and techniques for characterization, homology modeling and active site prediction of MMP25 protein. The first step includes retrieval of MMP25 protein sequence from UniProt KB database. The physiochemical properties were analysed by using ProtParam tool and the secondary structure was predicted by using GOR secondary structure prediction tool. Later the 3D structure was predicted by using SWISS-MODEL server and the model was validated by using PROCHECK method. Then the active site of MMP25 protein was predicted by using CASTp server. These active sites of the protein can be used for drug designing purpose.

II. MATERIAL AND METHODS

1. Retrieval Of Sequence

The protein sequence of MMP25 was retrieved from UniProtKB protein database (www.uniprotkb.org) and saved in FASTA file format in notepad along with its accession ID. UniProtKB is a protein

database which is freely accessible to the public and it contains the amino acid sequences of proteins [29].

2. Physicochemical Analysis

The physicochemical properties was analysed by using ProtParam analysis tool. The ProtParam tool calculates parameters such as amino acid composition, molecular weight, theoretical pI, instability index, aliphatic index and grand average of hydropathicity (GRAVY) [30].

3. Secondary Structure Prediction

The secondary structure was predicted by using GOR secondary structure prediction tool [31]. The GOR method was developed by Garnier-Osguthorpe-Robson in 1917. This method uses the concept of information theory for the prediction of secondary structures in proteins. The GOR method predicts alpha helix, beta sheets, turn or random coils secondary structure at each position by analysing the given sequences.

4. Homology Modeling And Model Validation

The MMP25 protein sequence was used for comparative homology modeling by using SWISS MODEL server. SWISS-MODEL is a fully automated protein structure homology modeling server to make the protein models accessible to all biotechnologist [32]. After modeling, the quality and validation of the model was carried out by PROCHECK method using PDBsum server [33].

5. Active Site Prediction

The active site was predicted by using CASTp (Computed Atlas of Surface Topography of proteins) server. CASTp provides an online resource for locating, delineating and measuring concave surface regions on three-dimensional structures of proteins.

III. RESULTS AND DISCUSSION

1. Sequence Retrieval

The protein sequence of MMP25 was retrieved from UniProt KB database and the sequence was saved in FASTA file format in notepad. The protein name, organism name, UniProt KB ID and sequence length were shown in table 1.

Table 1. Retrieval of sequence

Protein name	Organism name	UniprotKB ID	Sequence length
MMP25	Homo Sapiens	Q9NPA2	562

2. Physicochemical analysis

The physicochemical properties was analysed by using ProtParam tool and the results were enlisted in table 2. As per the table, the MMP25 protein is unstable and basic in nature. The total number of positively charged residues (Arg+Lys) was higher than the total number of negatively charged residues (Asp+Glu).

Table 2. Physicochemical parameters of MMP25 protein

Parameters	Value
Number of amino acids	562
Molecular weight	62554.02
Theoretical Pi	8.76
Total number of negatively charged residues (Asp + Glu)	58
Total number of positively charged residues (Arg + Lys)	63
Instability Index (II)	51.82
Aliphatic index	69.48
Grand average of hydropathicity (GRAVY)	-0.499

3. Secondary Structure Prediction

The secondary structure was predicted by using GOR method. The secondary structure elements like alpha helix, beta sheets, extended strand and random coils were enlisted in Table 3. As per the table, the percentage of coils in MMP25 protein was higher than the percentage of alpha helix and extended strand.

Table 3. Secondary Structure Prediction

Secondary Structure Elements	Number of residues	Percentage
Alpha helix	105	18.68 %
Beta sheet	0	0.00%
Extended strand	87	15.48 %
Coils	370	65.84 %

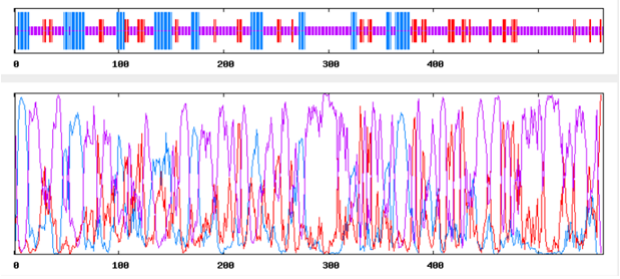


Figure 1. Secondary structure of MMP25 protein using GOR

4. Homology Modeling And Model Validation

The 3D structure was predicted by using the SWISS Model server. The template name with its identity score, template PDBID and validation scores were enlisted in Table 4. PDB id k076 was selected as template with 43.15% sequence identity. The model was visualized in RasMol as shown in figure 2. The quality and validation of the model was carried out by Ramachandran plot analysis using PDBsum server. Ramachandran plot analysis showed that the percentage of favoured region (89.4%) was higher than the percentage of additional allowed region (6.9%) concluding that the model were of reliable and good quality as shown in figure 4. The predicted three dimensional structure of MMP25 was deposited in Protein Model Database with PMDB id: PM0081482.

Table 4. Homology modeling and model validation

Parameters	Value
% of identity	43.15%
Template PDB ID	k076
Template Name	2mze.1.A
Validation score	89.40%

Figure 2. 3D model of MMP25 protein

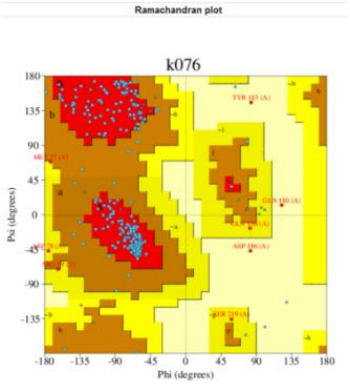
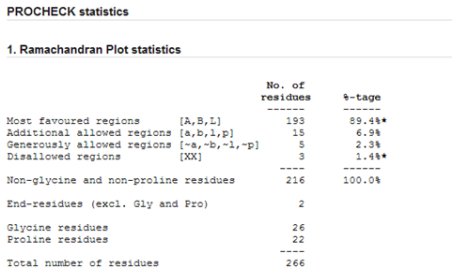


Figure 3. Ramachandran plot analysis



PROCHECK statistics

1. Ramachandran Plot statistics

		No. of residues	%-tage
Most favoured regions	[A,B,L]	193	89.4%*
Additional allowed regions	[a,b,l,p]	15	6.9%
Generously allowed regions	[~a,~b,~l,~p]	5	2.3%
Disallowed regions	[XX]	3	1.4%*
Non-glycine and non-proline residues		216	100.0%
End-residues (excl. Gly and Pro)		2	
Glycine residues		26	
Proline residues		22	
Total number of residues		266	

Figure 4. PROCHECK analysis

5. Active Site Prediction

The active site was predicted by using CASTp (Computed Atlas of Surface Topography of proteins) server. This server predicted that the MMP25 protein structure contains 135 binding sites.

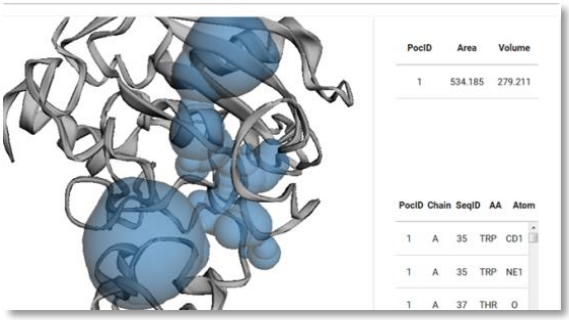


Figure 5. Active site prediction

IV. CONCLUSION

The present study mainly leads to the prediction of physiochemical and secondary structure of MMP25 protein using various in-silico tools and techniques. The primary structure indicates that the protein consists of 562 amino acids while the physiochemical properties conclude that the protein is unstable and basic in nature. The secondary structure predicts that the protein consist of high percentage of coils as compared to alpha helix and beta sheets. The 3D structure was modeled and the quality of the model was evaluated by PROCHECK and Ramachandran plot analysis. The active site of the protein was predicted by using CASTp server and this server predicts that it consists of 135 binding sites. From the present study it has been concluded that the MMP25 protein can be used as a target for the inhibition of airway remodeling in asthma disease by using in-silico drug designing methods.

V. REFERENCES

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