

Structural insights into the human gamma glutamyl transpeptidase-1 in the context of asthma

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INTRODUCTION

Asthma is a chronic respiratory disease involving inflammation and narrowing of the airways that is one of the major non-communicable diseases worldwide. It is a long-term inflammatory disease of the airways of the lungs. It is characterized by variable and recurring symptoms, reversible airflow obstruction and easily triggered bronchospasms. Symptoms include episodes of wheezing, coughing, chest tightness and shortness of breath. There are more than 300 million individuals that currently suffer from the disease and the prevalence continues to grow with each year, particularly in low and middle-income countries. Individuals of any age can suffer from asthma, but symptoms of the disease are most common in children. The prevalence of asthma varies widely in different regions of the world due to distinct genetic and environmental, occupational risk factors. However, this disparity appears to be closing as the prevalence in high income countries is reaching a plateau where as the prevalence in low and middle income countries continues to rise. Worldwide, it is estimated that approximately 334 million people currently suffer from asthma, and 250,000 deaths are attributed to the disease each year. The prevalence of the disease is continuing to grow, and the overall prevalence is estimated to increase by 100 million by 2025. Exposure to inhaled substances and particles that provoke an irritation or allergic reaction in the airways is a significant risk factor for the disease. Irritants may include dust, pollen, mould, smoke, chemical or pollution. Other triggers that can promote the presentation of asthma symptoms include cold air, anger, fear and physical exercise. Additionally, some medications such as non-steroidal anti-inflammatory drugs (NSAIDs) and beta blockers can trigger symptoms. Asthma has a comparably low mortality rate when contrasted with other chronic diseases. Poor control of asthma has been linked to inadequate access to medication used in the management of the condition. Asthma has no specific cure but management options are available to improve quality of life and allow patients to live an uninhibited life. Medications used to relieve symptoms or control inflammation and prevent exacerbation both have a role to play in managing asthma and improving overall outcomes of the disease. Environmental factors such as infections and exposure to endotoxins may be protective or may act as risk factors, depending in part on the timing of exposure in infancy and childhood. Some prenatal risk factors, including maternal smoking, have been firmly established, but diet and nutrition, stress, use of antibiotics and mode of delivery may also affect the early development of allergy and asthma. Later in childhood, putative risk factors include exposure to allergens, breastfeeding (which may initially protect and then increase the risk of sensitization), family size and structure, and sex and gender. In adulthood, recurrence of childhood asthma may be just as common as new-onset asthma, which may have an occupational basis. A better understanding of these risk factors may eventually lead to opportunities for primary prevention of asthma. γ -glutamyl transpeptidase (GGT1) has been implicated in an array of human diseases including asthma, reperfusion injury, and cancer. Inhibitors are needed for therapy, but development of potent, specific inhibitors of GGT1 has been hampered by a lack of structural information regarding substrate binding and cleavage. These are the first structures of any eukaryotic GGT with the cysteinylglycine region of the substrate-binding site occupied. These structures and the structure of apo-hGGT reveal movement of amino acid residues within the active site as the substrate binds. Asn-401 and Thr-381 each form hydrogen bonds with two atoms of GSH spanning the γ -glutamyl bond. Three different atoms of hGGT1 interact with the carboxyl-oxygen of the cysteine of GSH. Interactions between the enzyme and substrate change as the substrate moves deeper into the active site cleft. The substrate reorients and a new hydrogen bond is formed between the substrate and the oxyanion hole. Thr-381 is locked into a single conformation as an acyl bond forms between the substrate and the enzyme. These data provide insight on a molecular level into the substrate specificity of hGGT1 and provide an explanation for seemingly disparate observations regarding the enzymatic activity of hGGT1 mutants. This knowledge will aid in the design of clinically useful hGGT1 inhibitors.

EXPERIMENTALS

In the Protein Data Bank the number of structures obtained for asthma are 311 structures. A selected species of homo sapiens has been selected and the number of structures for asthma after applying the filter homo sapiens were 209. Then followed by opting the experimental methods, there are three major experimental methods that is X-RAY Diffraction method, Solution NMR and Electron microscopy and for the X-RAY Diffraction method 209 structures were obtained, for the Solution NMR method 8 structures were obtained and for the Electron microscopy method 2 structures were obtained and here the X-RAY Diffraction method has been obtained. The refinement solution filter at 1.5-2.0 Å has been applied where 57 structures were obtained. The protein structure 6XPB has been selected which is structure of human GGT1 complexed with 2-amino-4-(((1-((carboxymethyl)amino)-1-oxobutan-2-yl)oxy)(phenoxy)phosphoryl)butonic acid (ACPB) molecule. This structure belongs to the classification hydrolase, homo sapiens organisms and Komagataella pastoris expression system and there are no mutations. It consists of resolution 1.74 Å, where the R-Value work is 0.153 Å and R-Value free is 0.189 Å. Therefore to test whether the structure is of good quality we do two respective tests that is ;
Test:1- R-Free - R-Work: for a good X-ray crystal structure the difference between R-Free and R-Work should be less than or equal to 0.05 or 5%. For this structure : $0.189 - 0.153 = 0.036 = 0.036 * 100 = 3.6\%$.
Test:2- R-Work value should be roughly equal to 1/10th of Resolution value: $\text{Resolution}/10 = 1.74/10 = 0.174$.
Hence, the two tests are qualified therefore the structure is of good quality. It consists of two macromolecules that is Glutathione hydrolase 1 heavy chain and Glutathione hydrolase 1 light chain and five small molecules that is V7D, NAG, CL, NA. We used pymol software to analyze the structure.

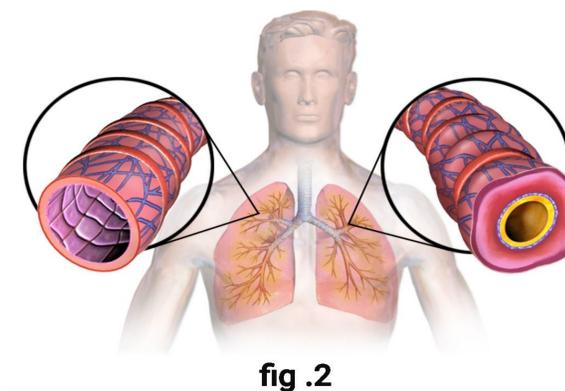


Fig-2 represents the diagram of asthma

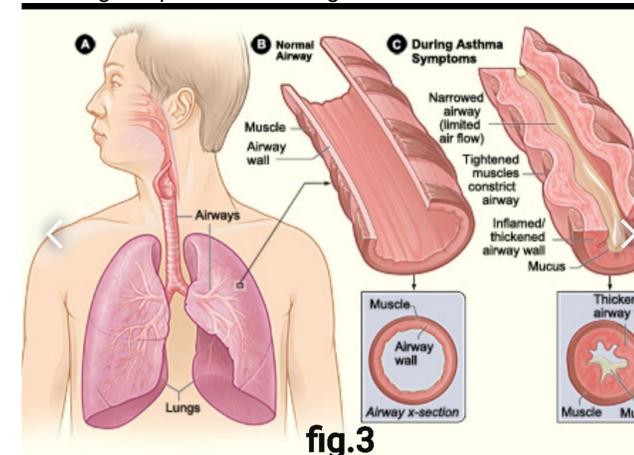


Fig-3 shows the location of the lungs and air ways in the body

RESULTS & DISCUSSION

After analyzing the structure in pymol two chains are obtained:
Green chain-chain A
Cyan chain-chain B
In chain A, Alpha helices are 13 whereas the Beta strands are 11.
In chain B, Alpha helices are 6 whereas Beta strands are 10. Therefore, we can analyze polar contacts for each ligand by using pymol.

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