Name of the Scholar : Poonam Singh
Name of the Supervisor : Mohamad Aman Jairajpuri
Department : Biosciences
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Abstract

Serine Protease inhibitors like antitrypsin, antichymotrypsin, C1-inhibitor, antithrombin and plasminogen activator inhibitor, play absolutely critical role in the control of proteinases, involved in the inflammatory, complement, coagulation and fibrinolytic pathways respectively, and are associated with diseases like emphysema/cirrhosis, angioedema, familial dementia, chronic obstructive bronchitis and thrombosis**.** The mechanism of inhibition of serpin requires large scale conformation change and native state of serpin is in a metastable state which transforms into a stable state when they inhibit target proteases. Serpins are prone to conformational diseases due to their susceptibility to undergo point mutations especially in mobile domains that can results in aberrant intermolecular linkage and polymer formation. The effects of such protein aggregation are cumulative, with a progressive loss of cellular function. Serpin polymerization is a significant problem and devising a cure has been cumbersome owing to their complex mechanism of inhibition, metastable nature, cofactor binding ability and large scale conformational change. Critical understanding of the factors and mechanisms promoting serpin misfolding and those regulating serpins conformational changes are essential for elucidating the etiology of serpin based diseases due to polymerization.

Comprehensive computational studies on the serpin superfamily are lacking most of the work done till date is with individual serpins. A collective study of serpin superfamily is critical to answer many question regarding their structure function and diseases and can help design cure. We have tried to analyze few aspect of this family computationally and show that it can give critical insight into their functional mechanism and defects. We took a dataset of diverse serpin with point mutations that are prone to polymerization and studied their location, burial, depth, stability and cavities. The data clearly shows the importance of residue burial linked shift in the conformational stability as a major factor in increasing the polymer propensity in serpins. Helix B in the shutter region is identified as a mutation hot spot that can lead to polymerization. Analysis shows that in most cases the amino acid involved in the polymerization was completely buried in the native conformation. However the cleaved and polymerized conformations of serpin showed the exposure at the N-terminal of helix B. Our data for the first time shows a plausible role of helix B in the mechanism of serpin inhibition and polymer formation across many inhibitory serpins.

Antithrombin variants didn't show strand 6B deformation and helix B exposure during inhibition. This prompted us to do an independent study of the antithrombin conformations and the results show the following. Structural overlap of alpha and beta antithrombin indicates that the differences between the two isoforms are in or near the heparin binding site. ASA analysis showed that drastic changes in residue burial were seen specifically in helix D and that the beta form of antithrombin seems to be in the activated conformation as compared to alpha based on the burial, cavity and hydrogen bond switch analysis of the helix D. Helix A is involved in the initial heparin binding interaction but progressively more helix D and N-terminal residues are involved in the later stages of antithrombin conformation change and inhibition mechanism. A hydrophobic cavity at the C-terminal of D-helix may play a critical role in the antithrombin activation mechanism. We showed critical involvement of the serpin cavities in polymerization and also in the isozyme specificity and function of antithrombin. This prompted us to do a detailed analysis of the serpin cavities of serpin like antitrypsin, neuroserpin, antithrombin, antichymotrypsin and plasminogen activator inhibitor, to find their area and volume and to assess if they are part of functionally and structurally important regions of protein. Largest cavity in the native state is centered around the shutter region in most of the serpin with the exception of antithrombin. Largest cavity in antithrombin was around helix D in a region that transforms the conformation change to the reactive center loop on account of heparin binding. In transition from native to polymerized or cleaved state most of the serpins showed massive increase in the largest cavities. Cavity filling variant are shown to retards polymerization by decreasing the size of its cavity. An evolutionary study clearly shows the regions in cavity which has been conserved during the course of evolution. It was hypothesized that increased polymerization propensity of the serpin shutter region variants is due to their presence in the large cavity in an area that is involved in conformational change. The conclusions strengthen the notion that cavity size and its variations may have critical role to play in the serpin inhibition and polymerization mechanism.

Previous evidence suggests that chemical chaperone can be promising in reducing the rate of polymerization in serpins but its binding affinity and interactions remains largely unknown. A molecular docking study was done using Autodock Vina with the objective of targeting the predicted cavities with chemical chaperones. We used amino acid, carbohydrate and methylamine based chaperones and predicted their best binding site and affinity. The results show that carbohydrate based chemical chaperone like sorbitol, sucrose, arabitol, and trehalose and amino acid based chaperones like DOPA, phenylalanine, arginine and glutamic acid are the most effective in binding serpins. Our results reaffirm that a shutter region cavity which invariably is the largest cavity in the native state of many serpins may be the ideal target to block polymerization. Most of these chemical chaperone interacted with residues in the shutter region and the helix D arm at the C-terminal which are part of the largest cavities.