Role of a Scaffold in the Inhibitory Process of a Serine Protease Inhibitor

Jiban K. Dattagupta, U. Sen, J. Dasgupta, S. Khamrui, *Crystallography and Molecular Biology Division, Saha Institute of Nuclear Physics, Kolkata-700064, India.* E-mail: jiban@cmb2.saha.ernet.in

The inhibitory loop of a serine protease inhibitor has a characteristic conformation while the remaining part of the molecule, known as scaffold, has widely different folds in different families of inhibitors. To understand the exact contribution of the 'inhibitor scaffold' towards the inhibition process, a classic β-trefoil fold protein, Winged bean Chymotrypsin Inhibitor (WCI), has been chosen. Owing to the crucial strategic location, as seen in our previous crystallographic and molecular dynamics studies, a scaffolding residue Asn14 has been targeted for mutagenesis by residues of different shapes and charges and the ability of chymotrypsin inhibition by the resulting mutants has been measured. Crystal structures of the mutants were determined and it was observed that the degree of loop deformation is inversely proportional to the extent of chymotrypsin inhibition.

Similarly, through mutations in the WCI loop region, two chimeric proteins are attempted with loops of trypsin inhibitors like ETI and STI on the scaffold of WCI. A comparison of binding constants of these chimeric proteins with their respective wild type ones can be used to understand whether the scaffold of WCI is best suited for chymotrypsin inhibition or it can be used for trypsin inhibition as well. As a first step towards this approach, we found that the single mutation (Leu 65→Arg) at P1 converts WCI to a potent inhibitor of trypsin with a K_i value comparable to ETI and STI indicating that the role of the scaffold of WCI is comparable to that of ETI and STI. Structure of this mutant (L65R) at 2.15Å provides a clue to this altered inhibition.

Keywords: serine-protease inhibitor, mutational analysis, inhibitor design