

Chickpea Kunitz Inhibitor: A mechanistic basis for trypsin inhibition

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Kunitz type trypsin inhibitor (KTI) family is one of the most versatile families of protease inhibitors. Their exact natural physiological role in plants is still unclear. According to some reports, KTIs are involved in proper growth and development of plants. Some researchers suggested their role in recovery from physical damage, while others have shown their applications in plant's defence from pests and pathogens. Some reports also suggest their efficacy against HIV proteases, cancer etc. Today, under Kunitz legume family in Pfam (PF00197), about 766 sequences under 124 plant species with 55 (9 native and 46 mutant) structures in PDB have been reported.

Chickpea is world's 3rd legume crop with India as a top producer in the world. Leguminous plants are known to have more than one isoforms/ variants of Kunitz inhibitors, yet very little is known about chickpea KTIs. From draft chickpea genome, we identified about 8 sequences that could fit into Kunitz family of which 2 have been already reported. Out of these 8 sequences, the one coding for an inhibitor TPI2, showed unique reactive loop sequence which is different from canonical Kunitz sequences. Also it does not contain conserved Arg65 (STI nomenclature) residue in the reactive loop. Thus the TPI2 was cloned into GS115 strain of *Pichia pastoris* and the secreted protein was purified to homogeneity. The protein was crystallized in P1 21 1 space group and diffracted at 1.9 Å resolution. Parameter for a= 38.74Å, b= 88.27Å and c= 59.82Å while α , β , γ were 90.00°, 108.89°, and 90.00° respectively.

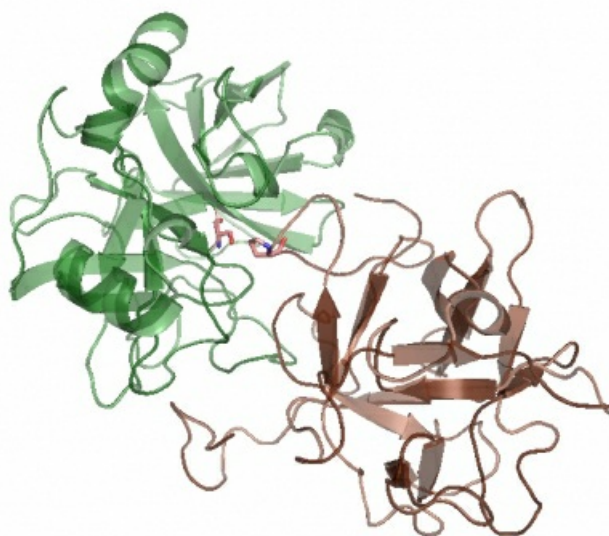
Our biochemical studies showed that TPI2 inhibits fungal proteases more effectively than insect gut proteases. Also previous reports hint towards the expression of TPI2 in roots instead of aerial plant parts.

Meanwhile we docked modelled TPI2 with structures of Trypsin from three different sources viz. *Fusarium oxysporum*, *Helicoverpa armigera* and bovine pancreas. Docked complexes were simulated using the NAMD2.8 simulation package2 with the CHARMM22 all-atom force field with CMAP correction. All complexes seemed stable which could be supported with preliminary biochemical assay using BApNA an artificial substrate for trypsin.

This collectively suggest that the Kunitz inhibitors in chickpea might be playing role in defense from soil borne pathogens. Further in detailed studies on structure would throw light on possible clues for differential reactivity towards trypsin from different sources

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[2] Wojciech R. Rypniewski, (1995), Acta Cryst., D51, 73-84



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