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[posters]

## Accepted Posters

### Structural Bioinformatics

Poster LB-28

**In vitro Molecular Evolution of Bean alpha-Amylase Inhibitors to Investigate Specificty of Binding to alpha-Amylases**

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**Short Abstract:** Combined DNA shuffling and Phage-display are important techniques to generate large number of alpha-amylase inhibitor mutants in vitro in order to identify new molecules with improved activities towards specific insects. We present new mutants and discuss possible repercussion of mutations on protein stability and function. We also introduced the STING parameter

**Long Abstract:**
Despite the presence of a family of defense proteins, containing phytohemagglutinin, arcelins and alpha-amylase inhibitors (alpha-AIs), domesticated varieties of the common bean, Phaseolus vulgaris can be attacked by insect-bruchids causing serious damage to stored grains. The two distinct active forms of alpha-AIs in P. vulgaris, alpha-AI-1 and alpha-AI-2 show different specificity against bruchids. Zabrotes subfasciatus alpha-amylase (ZSA) is inhibited by alpha-AI-2 but not by alpha-AI-1 inhibitor. In contrast, porcine alpha-amylase (PPA) is inhibited by alpha-AI-1 but not by alpha-AI-2. Previous study to understand the molecular basis of such specificity, involved constructions of the four mutants of alpha-AI-2 using molecular modeling dates and site direct mutagenesis (Silva et al., Prot. Eng.13:167, 2000; Silva et al., PAB 2004). Although those mutants containing substitutions and insertions of various parts of the binding interface lost their activity against ZSA no difference in activity was found towards PPA. Since the same N-terminal loop modifications (Ser and Tyr insertions on the position 32 and His33Asn substitution) were included in all mutants, the results showed that these amino acids alone were sufficient to completely abolish the inhibition in ZSA, but were not sufficient to change the specificity of the inhibitor towards PPA. In summary, the results indicated the need for a greater number of mutants to explain parameters involved with intricate specificity.
In this context the present research focused on the screening of alpha-AIs variant genes using phage display methodology from a previously generated alpha-AIs shuffled library. This library was produced by homologous gene recombination (DNA shuffling) of alpha-AI1 and alpha-AI2 from common and wild bean respectively, resulting in 3,7 x 107 recombined genes. The fusion phages selected by Z. subfasciatus alpha-amylases binding was enriched after 2nd selection cycle. The DNA phagemid from 2nd round was prepared to transform E. coli TOP10F' cells. 96 randomly picked IPTG-induced clones were tested in immunodots. The positive clones were sequenced and the translate aminoacid sequences compared to wild inhibitors showed 11 different mutants selected from Z subfasciatus alpha-amylases panning. To guarantee the correct folding needs to amylase inhibitory activities these variant genes were sub-cloning using plant expression vector to transform Arabdopsis thaliana. The total extracts of plants were used in vitro assays to select specific and potent alpha-amylase inhibitors against bruchids. Our data indicate variability among the toxicity of the recombinant proteins. Combined DNA shuffling and Phage-display are important techniques also constituting a strategy to generate large number of alpha-amylase inhibitor mutants in vitro in order to identify new molecules with improved activities towards specific insects. We present data on new mutants and discuss possible repercussion of mutations on protein stability and function. We also introduce the STING parameter "amino acid co-evolution" into our analysis of the function and stability determining residues.

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