Functional Characterization and Protein Engineering of Prolyl Endoprotease Neprosins from *Nepenthes rafflesiana*

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Background

Prolyl endoprotease (PEP) belongs to a serine protease family that cleaves protein at proline residues. Previous studies (*1-3*) showed PEP cleaves gliadins, a sub-component of gluten that cannot be digested by gluten-sensitive individuals, causing intestinal inflammation in celiac disease patients.

Methods

In this study, we aim to functionally characterize two novel PEPs found in the pitcher fluids of a carnivorous pitcher plant *Nepenthes rafflesiana*, namely neprosin-1 (Npr1) and its homolog, neprosin-2 (Npr2). These genes were cloned in pET-28(b)+ followed by protein expression using *Escherichia coli* (*E. coli*). The amino acid sequences of both neprosins were compared to other known PEPs and potential protein 3D-structures were generated using Iterative Threading Assembly Refinement (I-TASSER) and structurally aligned using Distance-matrix Alignment method (DALI).

Results

Sequence analysis indicates over 90% identity with a previously reported neprosin from *Nepenthes* × *ventrata* shown to degrade gliadins (*2*) . However, it showed low similarity with the PEP structures in the Protein Data Bank. The extraction of IPTG-induced cells grown at 16℃using glycine-HCl pH 2.5 buffer yielded soluble recombinant proteins at the expected size of 41.2 kDa and 39.4 kDa for Npr1 and Npr2, respectively. The His-tag recombinant proteins will be purified to homogeneity for further functional assays and protein crystallization. Mutagenesis of catalytic site will be performed for further protein engineering.

Conclusion

Recombinant plant-based neprosins will allow economical and efficient use in enzyme therapy to treat celiac disease and for the future development of a gluten-free wheat products.

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