

# Structural model for group-2 phytocystatin revealed the protein folds resembling to human latexin

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## Abstract

Tarocystatin (CeCPI), a group-2 phytocystatin, is a defensive protein of plants against phytopathogenic nematodes and fungi. The full length (FL) of this protein is composed of 205 amino acids, including N terminal (Nt) domain of 92 amino acids, C terminal (Ct) domain of 83 amino acids and an interconnected loop of 30 amino acids. Based on sequence alignment and secondary structure prediction, the overall folding of Ct domain resembles to the Nt domain which is one  $\alpha$ -helix followed four  $\beta$ -sheets. Thus, a structural model of FL of tarocystatin was proposed using homology modeling and protein-protein docking[1]. We also used the fold-recognition searching and got an interesting result that the structural model of CeCPI was similar to the structure of latexin[2], an endogenous protein inhibitor in the rat brain. However, the inhibitor activity of CeCPI was at the Nt domain rather than the latexin was at the Ct domain. It revealed the protein structure in group-2 cystatin belonged to two repetitive domains for stabilizing the overall structure, and showed the similar evolutionary trace in regulating the activity for proteinase inhibitors.

**Keywords:** CECPI, protease inhibitor, structural model, protein-protein docking, homology modeling

## 1 Introduction

Phytocystatins have been classified into three groups according to molecular weight (MW). Group 1, which MW ranged from 12 to 16kDa, was the smaller one and showed highly similarity to chicken egg white cystatin. The structure of group-1, a phytocystatin from rice named OCI [3], had been solved by NMR. Group-2 had MW around 23kDa and shared a conserved Nt domain with group-1. Group-3 contained several repetitive domains and showed a largest protein size in phytocystatin. Since group-2 phytocystatin had no any structures available, we firstly predicted the folding scheme for C-terminal domain using tarocystatin. The results showed the Ct domain was similar to Nt domain with one  $\alpha$ -helix followed four  $\beta$ -sheets. We further built the protein structure using homology modeling and combined these two domains using protein-protein docking, and also performed the searching the similar folding in the fold recognition server.

## 2 Method and Results

### *Homology modeling and protein-protein docking*

Based on the result from psiBLAST, the Nt and Ct domains of CECPI showed sequence similarity with OCI [3], then can be taken as a template for Ct structural prediction by modeler 8.1 [4]. The two structural domains are merged after analysis by the automatic docking system, Zdock2.3 [5], and then remodeled by modeler8.1 [4]. The final result was represented in Figure 1A.

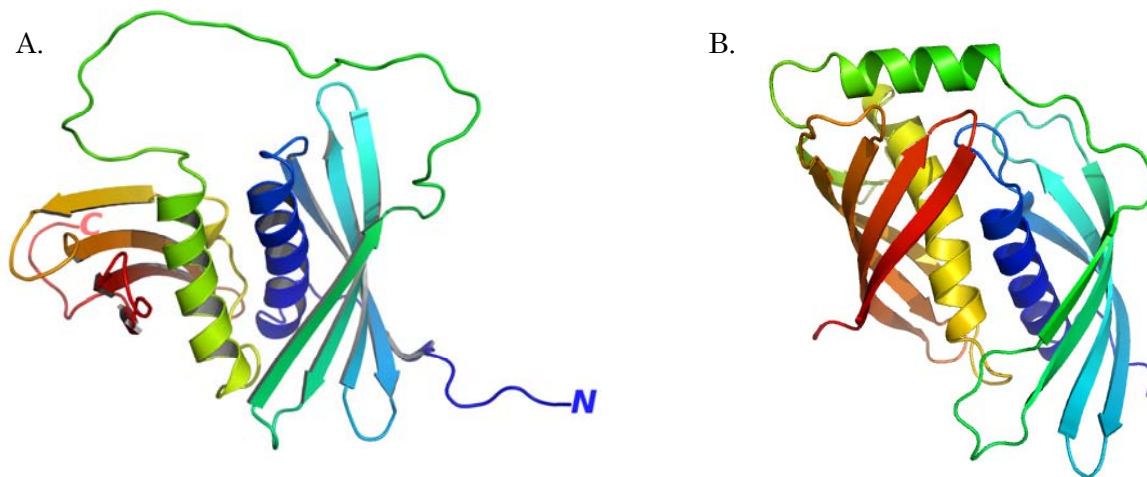


Figure 1: The structure resembling between CECPI (A) and Latexin (B). These two similar structures implied that the inhibitors of cysteine proteinase and carboxypeptidase had the similar folding with one  $\alpha$ -helix followed four  $\beta$ -sheets, and further combined another similar folding with no inhibitor function of Nt domain or Ct domain for enhancing the structural stability.

### 3 Discussions

Group-2 phytocystatin from taro corms showed the anti-papain activity, and may involve in regulating protein turnover rate. However, the group-2 phytocystatin contained additional Ct regions with about 100 amino acids than group-1. What is the role in the Ct domain of the group-2? From the sequence analysis of Ct region, we proposed the folding of Ctl would be similar to Nt domain. The Group-2 phytocystatin comprised of these two domains. The overall structure of group-2 phytocystatin was built using homology modeling and protein-protein docking. For further survey, group-2 phytocystatin showed similar folds with the crystal structure of a protein inhibitor of carboxypeptidase named latexin from rat brain. This is first report to demonstrate the structural resemblance between group-2 phytocystatin and the inhibitor of carboxypeptidase A4.

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