Complex Structure of Tarocystatin with Papain and Characterization of Tarocystatin Proposed the Role of C-terminal Extension for Inhibitory Mechanism of Group-2 Phytocystatins

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CeCPI-1 belongs to group-2 phytocystatins with 205 residues (Fig. 1). The N-terminal CY domain has been characterized with the mainly inhibitory ability of tarocystatin against papain, but the C-terminal CY-like domain didn't show any inhibitory activity against papain. Several lines of evidence have been proposed that the existence of C-terminal extension might have two possible roles: 1) The C-terminal extension of tarocystatin showed a weak papain activation properties; 2) A SNSL motif in C-terminal domain of barley HvCPI-4 showed the inhibitory activity to legumain. In this report, the full length of CeCPI-1 (FL) or the C-terminal extension of CeCPI-1 (CtD), respectively, in complex with the active form papain have been determined. In the complex of FL-papain, only the structure of N-terminal domain of CeCPI-1 (NtD) in complex with papain could be built at a resolution of 2.03 Å (Fig. 2A). The other structure of the remnant residues of CtD in complex with papain was refined to 2.6 Å (Fig. 2B). This first complex structure of phytocystatin-papain gave the information to observe the binding mode of phytocystatin-papain, and discovered the role of C-terminal extension for group-2 pytocystatins.

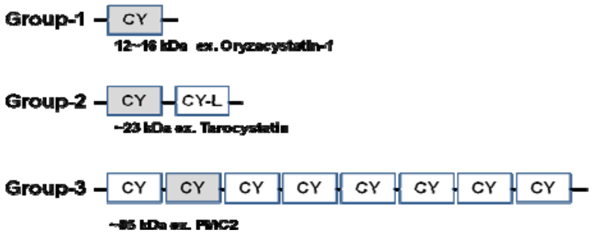

Fig. 1: Phytocystatins can be divided into three groups based on the molecular weight: Group-1 is around 12-16 kDa with one CY domain; Group-2 is around 23kDa with one CY domain and an extended CY domain at the C-terminus; Group-3 consisted of several repetitive cystatin domains with 85 kDa. The resolved structures of cystatin domain have been shaded by grey box.

The protease-inhibitor mixture of tarocystatin (FL) with papain was prepared for cocrystallization with a 1:1 molar ratio in concentration. After one month, the crystals of FL-papain were grown in an orthorhombic space group of P212121 with the cell dimension a=36.06Å, b=99.72Å, c=165.59, α=β=γ=90°. The complex structure of FL-papain was further resolved by molecular replacement method using the crystal structure of papain as a search model (pdb id: 1PPN). Two peak values were given in the cross rotation function and translation, and gave a 41.1% of R-factor by initial rigid body refinement. After model rebuilding and iterative refinement, the complex structure gave 18.1% of R-factor and 23.2% of Rfree at a resolution 2.03Å.

From the refined complex structure of FL-papain, only the N-terminal CY domain (NtD) of tarocystatin in complex with papain could be built, and no continuous electron density for C-terminal extension of tarocystatin could be observed. Finally, there are two complex structures of NtD with papain in an asymmetric unit, including two papain molecules (chain A and C: residues 1-212), two NtD of tarocystatin molecules (chain B: residues 2-9 and residues 16-91; chain D: residues 2-9 and residues 16-92), three acetic acid molecules and 576 water molecules.

The complex structure of chain A (papain) and chain B (NtD) is almost identical to the one of chain C (papain) and chain D (NtD) with a RMSD of 0.39 Å. The buried surface between NtD and papain is 2080 Å2. There are nine hydrogen bonds in the interface (chain B to chain A): Met4 (N) to Gly66 (O), Met4 (O) to Gly66 (N), Met4 (SD) to His159 (N), Gly5 (N) to Asp158 (O), Gly5 (N) to OCS25 (OD2), Gln49 (NE2) to Cys63 (O), Val51(O) to Trp177 (NE1), Ser52 (N) to Gly20 (O), Ser52 (OG) to Asn18 (OD1). One salt bridge is Glu18 (OE2) to Lys139 (NZ) (Fig. 2C). Cys25 in the papain structure is oxidized with three oxygen atoms to γS which can be observed by clear electron density (Fig. 2D).

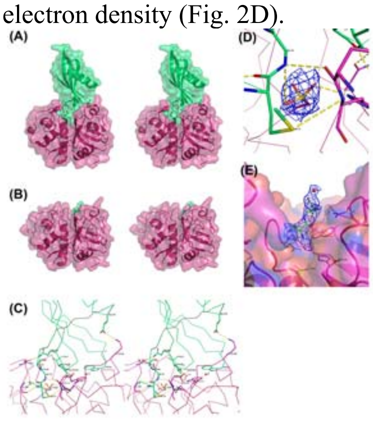

Fig. 2: (A) Stereo view of tarocystatin with papain complex structure. The remained NtD of Tarocystatin is colored by limegreen and papain is colored by warmpink, respectively. (B) The remnant residues of CtD colored by limegreen in complex of papain colored by warmpink. (C) Hydrogen bonding network between tarocystatin and papain. The residues of tarocystatin from residue 10 to 15 are not observed and are represented by black dots. (D) The 1σ of 2Fo-Fc electron density map for the oxidized sulfhydryl group of CYS-25 of papain. (E) The dipeptides, SER-ASN, of CtD are contoured by 1σ of 2Fo-Fc electron density map.