

Molecular Characterization of Three Cysteine Proteinase Inhibitors from Chinese Cabbage

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Objective

Three cDNA clones encoding phytocystatins from the flower buds of Chinese cabbage (*Brassica campestris* L. ssp. *pekinensis*), designated BCPI-1, -2, and -3 (*Brassica Cysteine Proteinase Inhibitor-1*, -2, and -3), were classified as belonging to two phytocystatin subgroups based on their carboxyl-terminal regions. To study the biochemical properties of three BCPIs, we assayed their inhibition of papain activity and the stability of the recombinant proteins.

Materials and Methods

1. Material: protein expression vectors (pRSET -A/-B), BANA (-N-benzoyl-DL-arginine-2-naphthylamide), -dimethylaminocinnamaldehyde, *E. coli* strain-BL-21 (pLys E).
2. Methods: Each of recombinant BCPI was purified from soluble fraction in the cytoplasm of BL-21 (pLysE). The pH and thermal stability of recombinant BCPI proteins were analyzed by measuring the loss of papain activity after incubating at various pH and temperature.

Results and discussion

BCPI-1 and -2 were identified both as the monomeric (32 and 33 kDa) and dimeric forms (72 and 73 kDa). The monomeric forms of BCPI-1 (K_i ; 68.4 nM) and -2 (K_i ; 67.7 nM) efficiently inhibited papain than that of the dimeric forms of BCPI-1 (K_i ; 101 nM) and -2 (K_i ; 99.8 nM). The both forms in BCPI-1 and -2 were unstable in the pH range of 7.0 - 11.5 (K_i ; 161 ~ 301 nM) but when the pH was changed into 4.0, the pH stability was maximized (K_i ; 99.8 and 101 nM). Furthermore, the low inhibitory activity of these proteins at pH 8.0 was

gradually increased with temperature increase. Once activated, the pH- and temperature-dependent behaviors of BCPI-1 and -2 were irreversible. However, BCPI-3 had a monomer of 16 kDa, K_i value of 63 nM against papain and was highly stable over a wide range of pH (K_i ; 63 nM) and temperature (K_i ; 63 nM). Differences in inhibitory activity and protein stability against papain found for the BCPIs suggest that the possibility of their differential physiological roles by different interactive mechanism of interaction between cysteine proteinases and BCPIs in Chinese cabbage.

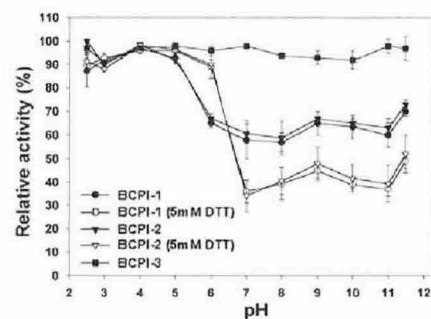


Fig. 1. pH stability profiles of the recombinant BCPIs.

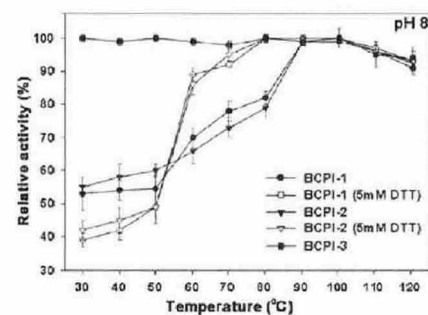


Fig. 2. Thermal stability of the recombinant BCPIs at pH 8.0.