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Partial characterization of a cysteine protease from *Panonychus citri*

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Abstract

A cysteine protease activity of *Panonychus citri* was detected and partially characterized. Proteolytic activity of the enzyme was enhanced by the addition of reducing agent, dithiothreitol (DTT), and also it was absolutely inhibited by cysteine protease specific inhibitors, such as trans-epoxy-succinyl-L-leucyl-amido (4-guanidino) butane (E-64) and iodoacetic acid (IAA). The other specific inhibitors of serine- and metallo-proteases could not inhibit the enzyme activity. This result showed for the first time cysteine protease activity of P. citri. Further studies on the biological roles of the enzyme are required.

Key words: *Panonychus citri*, cysteine protease

Citrus red mite, P. *citri* is known as a causative agent of asthma and rhinitis in citrus farmers at Jeju Island (1, 2). Allergic reactions of the mite are well documented, and it had been reported that major group 1 allergen (Der p 1) of house dust mite, *Dermatophagoides pteronyssinus* was identified as 30 kDa cysteine protease (3).

Parasites proteases play important roles in tissue penetration and nutrient uptake as well as evasion from immune attack in their host (4, 5). In addition, two cysteine proteases having molecular weight of 28, 27 kDa from lung fluke, *Paragonimus westermani* metacercariae exert critical roles in metacercarial excystment (6). However, cysteine protease of citrus red mite is not reported elsewhere until now.

In this study, the authors detected cysteine protease from crude extract of P. citri and partially purified, characterized its biochemical properties.

10 mg of citrus red mite *P. citri* were homogenized in a Teflon-pestle homogenizer with 20 mM sodium acetate buffer (pH 6.4) and the homogenate were centrifuged at 15,000 rpm for 40 min. The resulting supernatant was

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used as crude extracts and all procedure was done at 4℃.

Assays of proteolytic enzymes were measured using synthetic dipeptide substrates by the modified method of Chung et al (7). The used substrates were carbobenzoyl-phenylalanyl-arginyl-7-amino-4-methylcoumarin (Cbz-Phe -Arg-AMC) with 2 mM DTT for cysteine protease and succinyl-leucyl-leucyl-valyl-tyrosine-7-amino-4-methylcoumarin (Suc-Leu- Leu-val-Try- AMC) for serine or neutral protease. The inhibitor assay was performed with specific protease inhibitors such as IAA (20 μM), E-64 (10 μM) for cysteine protease, di- isopropylfluoro- phosphate (DFP, 2 mM) for serine- and 1,10- phenan- throline (2 mM) for metallo- protease.

For the partial purification of cysteine protease, the crude extract was loaded onto DEAE-Trisacryl M anion exchanger column (1.6 x 2 cm) previously equilibrated with 20 mM sodium acetate buffer (pH 6.4). The column was washed with the same buffer and absorbed proteins were eluted with step-wise increasing of NaCl molarity up to 0.5 M. Enzyme active fractions were pooled and concentrated, and then. analyzed purity by 7.5-15% gradient sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

The substrates specificity of crude extracts of citrus red mite showed that it selectively cleaved more Cbz-Phe-Arg-AMC than Suc-Leu-Leu-Val-Tyr-AMC. It seems likely that the crude extracts of the mite contained cysteine and serine protease, however, cysteine protease was more active than serine protease in this result (Table 1).

When the crude extracts were loaded onto DEAE-anion exchanger column, most protease was slightly absorbed and eluted at 0.05M NaCl. The fraction was dialyzed against deionized water and concentrated, and it was used as partially purified fraction. Further purification did not performed since available crude extracts and DEAE column fraction were very limited.

SDS-PAGE study showed that crude extracts were constituted with major protein bands including 70, 53, 32, 23 kDa and 14 kDa (Fig. 1). The unbinding fraction of DEAE column contained 32, 14 kDa protein bands and it showed a little proteolytic activity. The highly active fraction was consisted of 32, 24, 14 kDa protein bands and 24 kDa protein band appears to be cysteine protease (Fig. 1, lane 3). However, further purification would be required for precise molecular weight of the protease.

In inhibitors study, the partially purified enzyme was absolutely inhibited by cysteine protease specific inhibitors such as IAA and E-64. On the other hands, serine- or metalloprotease inhibitors including DFP and 1,10- phenanthroline could not affect the activity of the enzyme (Table 2). These results indicated that the purified enzyme belongs to the cysteine protease family. In our study, however, the detection and properties of other serine- or metalloprotease will be required on further study. In addition, the relationship between the cysteine protease and its allergenic property might be required on-going study.

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Table 1. Substrates specificity of crude extracts from *Panonychus citri*

Substrates	Activity of crude extracts*
Cbz-Phe-Arg-AMC	10.3
Suc-Leu-Leu-Val-Try-AMC	0.6

* The proteolytic activities were expressed as unit/ml and measured against each substrates after 1 hour incubation.

Table 2. Relative activities of partially purified cysteine protease by various inhibitors

Inhibitors	Relative Activity (%)
Control (without inhibitor)	100
Control (without DTT)	38.3
IAA	1.6
E-64	0.9
DFP	124.1
1,10-phenanthroline	65.1

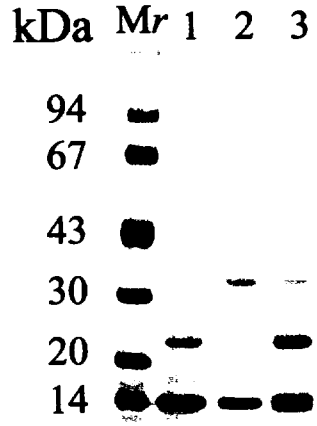

Fig. 1. SDS-PAGE of partially purified cysteine protease od *p. citri* Mr, standard marker proteins. Lanes 1, crude extracts; 2, unbound fraction through DEAE-column; 3, partially purified cysteine protease through 0.05M fraction of DEAE-column