

[SHORT COMMUNICATION]

IS A TRYPSIN-LIKE PROTEASE OF MITES A *Der f III* ALLERGEN ?

Tohru Ando, Reiko Homma, Yoshitaka Ino, Goro Ito, Atsushi Miyahara,
Hiroshi Yamakawa and Masahiro Iwaki

Section of Allergology, Research Laboratories, Torii & Co., Ltd.

Yasushi Okumura

Central Research Laboratories, Asahi Breweries, Ltd.

Matsunobu Suko, Michiko Haida and Hirokazu Okudaira

Department of Medicine and Physical Therapy, Tokyo University

A trypsin-like protease was purified from mite (*Dermatophagoides farinae*) fecal extract. In SDS-PAGE, the mite trypsin-like protease showed a single band at 34 kD. The purified trypsin-like protease possessed potent allergenic activity. Both the twenty N-terminal amino acid sequence and the amino acid composition of the purified protease were very similar to those of *Der f III*. These data strongly suggest that the trypsin-like protease in the mite is a *Der f III* allergen.

Key words: allergen — *Der f III* — *Dermatophagoides farinae* — trypsin-like protease

INTRODUCTION

Two species of mites, *Dermatophagoides pteronyssinus* (*Dp*) and *Dermatophagoides farinae* (*Df*) have been reported to be the most important sources of allergen in house dust^{1,2}. These species of mites have been intensively studied and, at present, two groups of major allergens, *Der I* group (*Der p I*, *Der f I*) and *Der II* group (*Der p II*, *Der f II*), have been identified by several groups³. Recently, we reported on a cysteine protease in extract from the body of the mite (*Df*) and identified that mite cysteine protease as the *Der f I* allergen^{4,5}. Chua et al. also reported that *Der p I*'s cDNA sequence had about 20% homology with several cysteine proteases⁶. Furthermore, Heymann et al. isolated the third mite allergen, *Der f III* and showed that the N-terminal amino acid sequence of *Der f III* was similar to that of serine

proteases such as bovine trypsin⁷. In addition, a serine protease was purified from *Df* extract⁸. We also purified a trypsin-like protease which belonged to the serine proteases.

This paper describes the purification of this trypsin-like protease of *Df* and presents evidence that this protease is actually a *Der f III* allergen.

MATERIALS AND METHODS

Mite fecal extract

D. farinae was cultivated in a flat type flask containing animal food at 25°C and 75% humidity for 1 month. After cultivation, feces were collected by sieving (250 mesh). Feces-rich material (32.7 g) was extracted for 30 min in 655 ml saline and centrifuged (10,000 × g for 60 min at 4°C). After the supernatant was filtered through a membrane filter (pore size 0.45 μm), the filtrate was used as the mite fecal extract (MFE).

Purification of mite trypsin-like protease

MFE (655 ml) was concentrated to 200 ml using polyethylene glycol (M.W. 50 kD), dialyzed against 0.02 M phosphate buffer (PB), pH 6.0 and applied to a DEAE-Sephacel (Pharmacia) column equilib-

Received: February 13, 1992

Accepted: April 2, 1992

Abbreviations: Boc t-butyloxycarbonyl; ELISA enzyme-linked immunosorbent assay; kD kilodalton; MCA 4-methyl-coumaryl-7-amide; MFE mite fecal extract; MW molecular weight; PB phosphate buffer; SDS-PAGE sodium dodecyl sulfate-polyacrylamide gel electrophoresis

rated with the same PB. Elution was performed with a linear gradient concentration of NaCl from 0 to 0.4 M with the same buffer. The 0.15 M NaCl eluted fractions containing the potent trypsin-like protease activity were pooled, concentrated to 10.1 ml by polyethylene glycol and the fraction by ammonium sulfate precipitation at 60–80% saturation was collected. After being dissolved in 1 ml of 50 mM PB, pH 7.4, the sample solution was applied to a column of Superdex 75 pg (Pharmacia) and the fractions containing the potent protease activity were pooled.

Der f I and *Der f II* were purified by column chromatography as reported previously^{5,9)}.

Measurement of protease activity

A synthetic substrate of trypsin (Boc-Phe-Ser-Arg-MCA) was purchased from the Peptide Institute, Inc. (Osaka, Japan). The protease activity was determined by the method described previously¹⁰⁾.

Measurement of allergenic activity

Measurement of allergenic activity was performed according to the method of fluorometric ELISA reported by Sakaguchi et al.¹¹⁾. Briefly, a microplate (Dynatech, Immulon II®) was coated overnight with 0.05 ml of purified protease, *Der f I* and *Der f II* in 0.1 M carbonate buffer, pH 9.5, and blocked with 3% gelatin in PB for 1 hr. After washing, 0.05 ml of fivefold-diluted sera from 23 mite allergic patients all diagnosed as asthma (13 male and 10 female, aged 16 to 65 years with a positive prick test to *D. farinae*) were added to the wells. After 3 hr incubation, the plate was washed and incubation was performed with 0.05 ml of tenfold-diluted β -D-galactosidase-conjugated anti-human IgE (Phadezyme kit®, Pharmacia) for 16 hr. The microplate was washed and 0.1 ml of 0.1 mM 4-methyl-umbelliferyl β -D-galactoside was added. After incubation for 2 hr, the enzyme reaction was stopped by adding 0.1 ml of 0.1 M glycine-NaOH, pH 10.3, and the fluorescence of 4-methyl-umbelliferone released from the substrate was measured at excitation (365 nm), and emission (450 nm), on a fluorometer. All incubations were performed at 25°C.

Amino acid sequencing of mite trypsin-like protease

The amino acid sequencing of the purified mite trypsin-like protease was done with a gas-phase protein sequencer (Model 471A, Applied Biosystem Co.)¹²⁾.

RESULTS AND DISCUSSION

A mite trypsin-like protease in the MFE of *D. farinae* was purified by ammonium sulfate precipitation and chromatography on a DEAE-Sephacel and Superdex 75 pg. The purified protease gave a single band of protein at MW 34 kD on SDS-PAGE under reducing conditions (data not shown). The specific activity of the purified mite trypsin-like protease was 13.5 times greater than that of MFE. The allergenic activity of the purified mite trypsin-like protease was measured using 23 mite allergic patients' sera. As indicated figure 1, mite trypsin-like protease as well as *Der f I* and *Der f II* caused reactions in over 70% of the patients' sera, and the level of reactivity differed from individual to individual. These data suggest that mite trypsin-like protease may be one of the major mite allergens. Recently, Takahashi et al. purified trypsin-like protease and it named *Df* protease⁸⁾. They pointed out the similarity between *Df* protease and *Der f III* by comparing N-terminal amino acid sequences of *Df* protease with those of *Der f III*¹³⁾. However, the N-terminal amino acids sequences did not completely agree with each other, and the authors did not measure the allergenic activity of *Df* protease against atopic patients' sera. As mentioned above, our mite trypsin-like protease has potent allergenic activity. We measured twenty N-terminal amino acid sequences of our mite trypsin-like protease. As shown in figure 2, the N-terminal amino acid sequence of mite trypsin-like protease was very similar to those of *Df* protease and *Der f III*. Furthermore, the amino acid composition of our mite trypsin-like protease was also similar to that of *Der f III* (data not shown).

These results strongly suggest that mite trypsin-like protease is a *Der f III* allergen itself.

In addition, this protease might be associated with the mite's digestive process as the trypsin-like protease activity of mite fecal extract was

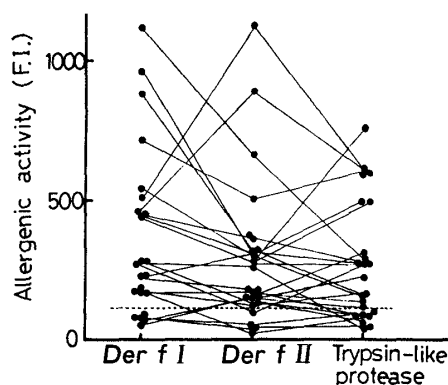


Fig. 1. The allergenic activity of trypsin-like protease to 23 mite-allergic patients' sera. The allergenic activity of the mite trypsin-like protease was measured by fluorometric ELISA. The cut off value is 100 F.I. (fluorescence intensity).

N-terminal amino acid sequence	
	1 20
Trypsin-like protease	I V G G V K A Q A G D • P Y Q I • L Q •
Df-protease	I V G G V K A Q A G D • P Y Q I S L Q S
Der f III	I V G G V K A L A G D • P Y E I S L E V
Hornet chymotrypsin	I V G G T D A P R G K Y P Y Q V S L R A
Crayfish trypsin	I V G G T D A V L G E F P Y Q L S F Q E
Bovine trypsin	I V G G Y T C G A N T V P Y Q V S L N S

Fig. 2. N-terminal amino acids sequence of trypsin-like protease, Df protease, Der f III and other serine proteases.

*not detected.

about 1800 times stronger than that of mite body extract.

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ダニのトリプシン様蛋白分解酵素は *Der f III* アレルゲン？

島居薬品株式会社研究所

安藤 徹 本間玲子 猪 好孝 伊藤吾朗

宮原 淳 山川洋志 岩城正廣

アサヒビール（株）中央研究所応用技術研究所

奥村 康

東京大学医学部物療内科

須甲松伸 灰田美和子 奥平博一

コナヒョウヒダニの排泄物抽出液からトリプシン様蛋白分解酵素を精製した。精製トリプシン様蛋白分解酵素の分子量は、SDS-PAGE で34kD であり、またこのものは強いアレルゲン活性を有していた。トリプシン様蛋白分解酵素と *Der f III* の N 末端アミノ酸20残基およびアミノ酸組成は極めて類似していた。

以上のことから、ダニのトリプシン様蛋白分解酵素は、ダニの *Der f III* アレルゲンであることが強く示唆された。