

[SHORT COMMUNICATION]

CLONING AND SEQUENCING OF cDNAS CORRESPONDING TO
MITE MAJOR ALLERGEN *Der f* II

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A cDNA library corresponding to mite protein was screened employing anti-*Der f* II antibody. Two possible clones were obtained, which contained plasmids, pFL1 and pFL11, respectively. Both plasmids had insertions of about 500 base pairs. The DNA sequences of the two insertions were determined, from which the amino acid sequences were deduced. The amino acid sequence of the purified native *Der f* II protein could be determined to 45 residues from the N-terminus. As a result of comparison, we concluded that the cDNAs prepared from live mite *Dermatophagoides farinae* corresponded to the mite allergen, *Der f* II.

Key words: cDNA — cloning — *Der f* II — sequence

INTRODUCTION

It is well known that house dust is one of the most important allergens causing atopic asthma and other atopic diseases¹⁾. Although house dust contains various kinds of allergens such as mites, animal danders and fungal spores, mites have been proposed as a major causative allergen in many atopic diseases²⁾. A large amount of purified mite allergen is necessary for diagnostic and therapeutic purposes. However, preparation of the purified allergen on a large scale is, at present, practically impossible. Therefore, we have attempted production of the purified major allergen of *Dermatophagoides farinae*, *Der f* II, by employing recombinant DNA techniques; that is, cloning and sequencing the cDNA corresponding to *Der f* II.

MATERIALS AND METHODS

Mites

D. farinae was cultivated in a flat type flask

containing animal feed at 25°C and 75% humidity for 30 days. After cultivation, live mite bodies were harvested by filtration as described previously³⁾.

Antisera

Rabbit antisera to *Der f* II⁴⁾ was kindly supplied by Dr. Yasueda (National Sagamihara Hospital, Kanagawa, Japan).

Preparation and screening of cDNA library to D. farinae

Five grams of live mites were rapidly frozen with liquid nitrogen. Purified mRNA from the mites was prepared by guanidine isothiocyanate extraction and oligo (dT) cellulose column chromatography (RNA extraction kit, RPN. 1264; Amersham International plc., mRNA Purification Kit, 27-9258-01; Pharmacia LKB Biotechnology AB). The cDNA library was prepared according to the manufacture's directions (cDNA synthesizing system plus, RPN1256Y; Amersham International plc.).

The cDNA library was expressed in *Escherichia coli* MC1061 employing an expression vector pUEX1⁵⁾ (cDNA cloning system-plasmid pUEX1, RPN. 1282; Amersham International plc.) according to the manufacturer's instructions.

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The clones were screened by colony immunoassay employing rabbit anti-*Der f* II antibody and an immunoscreening system (Super Screen immunoscreening system, RPN1281Z; Amersham International plc.). Two possible candidates were found among 1,600 clones. The plasmids of the clones were named pFL1 and pFL11, respectively.

Sequencing of cDNA

Two kinds of plasmid DNAs were isolated from the two candidates (No. 1 and No. 11) respectively. The cDNA insert was excised by *Bam*HI. *Bam*HI-cDNA fragments were digested with various restriction endonucleases such as *Nco*I. The subcloning was carried out employing the plasmids pUC118 and pUC119⁶⁾. After isolation of transformed *E. coli* JM105, the plasmid DNA was recovered, from which a single strand DNA was prepared. DNA sequencing was carried out by the method of Sanger⁷⁾.

Sequencing of amino acid of native *Der f* II protein

Der f II protein from *D. farinae* was purified by column chromatography as reported previously⁸⁾. The final purification was accomplished by HPLC (Model 130A, Applied Biosystems Co.; Column, C₈ 300A (2.1 ID × 30 mm); buffer A, 0.1% trifluoroacetic acid (TFA) in H₂O; buffer B, 0.1% TFA in 70% acetonitrile; gradient, 0% buffer B to 100% buffer B at 2.2% per minute; flow rate, 200 µl/min). Determination of the amino acid sequence of the purified *Der f* II was carried out employing a gas phase protein sequencer (Model 471A, Applied Biosystems Co.).

Identification of the antigenicity of the fused *Der f* II

E. coli transformed by the plasmid pFL1 was cultured in L-broth with 50 µg/ml of ampicillin. The incubation was carried out at 30°C until the optical density at 660 nm of the culture reached 0.6. Then, the cultivation temperature was raised to 42°C and the culture was incubated for a further 2 hours. The cells were harvested by centrifugation. A sample from the cell was prepared by extraction at 100°C for 5 minutes in the buffer containing 20% (v/v) glycerol, 20% (v/v) 2-mercaptoethanol, 6% (w/v) sodium dodecyl sulfate (SDS) and 0.125 M Tris-HCl (pH 6.8). The sample was subjected to SDS-polyacrylamide gel

electrophoresis. The gel used was 4~20% gradient gel. After electrophoresis, part of the gel was stained by Coomassie Brilliant Blue and the rest was subjected to Western blotting⁹⁾. The proteins transferred onto a nylon membrane were reacted with rabbit anti-*Der f* II antibody, washed, and were detected by peroxidase-labeled anti-rabbit IgG antibody.

RESULTS

Selection of the clone containing *Der f* II gene

The cDNA library prepared from *D. farinae* was inserted into an expression vector pUEX1, and then *E. coli* MC1061 was transformed by the recombinant plasmids. Among 1,600 transformants, two clones, No. 1 and No. 11, appeared to be the objective clones employing colony immunoassay with anti-*Der f* II antibody. Each plasmid was recovered from the clones. The isolated plasmids, pFL1 and pFL11, were analyzed by agarose gel electrophoresis. Both plasmids had approximately 500 base pairs insertion at the end of the β -galactosidase gene. Therefore, the foreign proteins were fused to β -galactosidase. On the other hand, the proteins produced by clones No. 1 (pFL1) and No. 11 (pFL11) were determined by Western blotting employing anti-*Der f* II antibody. Both clones produced an antibody-binding component with a molecular weight of about 130,000, while the host transformed by pUEX1 without any insertion did not produce such a protein.

Sequencing of cDNA

The cDNA sequences and the deduced amino acid sequences from the cDNA inserts of pFL1 and pFL11 are shown in Figs. 1 and 2, respectively. There were minor differences observed between the two sequences. That is, three amino acid residues were different from each other at positions 89, 101 and 124.

Amino acid sequence of native *Der f* II

The native *Der f* II purified by HPLC was subjected to determination of its amino acid sequence. Forty five amino acid residues from N-terminus could be determined (data not shown). This sequence was identical to those of the se-

Adaptor			
1	GGT ACC ATG GTT TCA TTG TTG GTA GCA GCC	30	
1	Gly Thr Met Val Ser Leu Leu Val Ala Ala	10	
31	GTT GTT GCC GAT CAA GTC GAT GTT AAA GAT	60	
11	Val Val Ala <u>Asp</u> <u>Gln</u> Val <u>Asp</u> Val <u>Lys</u> <u>Asp</u>	20	
61	TGT GCC AAC AAT GAA ATC AAA AAA GTA ATG	90	
21	(Cys) <u>Ala</u> <u>Asn</u> <u>Asn</u> <u>Glu</u> <u>Ile</u> <u>Lys</u> <u>Lys</u> Val Met	30	
91	GTC GAT GGT TGC CAT GGT TCT GAT CCA TGC	120	
31	Val <u>Asp</u> <u>Gly</u> (Cys) <u>His</u> <u>Gly</u> Ser <u>Asp</u> Pro(Cys)	40	
121	ATC ATC CAT CGT GGT AAA CCA TTC ACT TTG	150	
41	<u>Ile</u> <u>Ile</u> <u>His</u> <u>Arg</u> <u>Gly</u> <u>Lys</u> Pro <u>Phe</u> Thr Leu	50	
151	GAA GCC TTA TTC GAT GCC AAC CAA AAC ACT	180	
51	<u>Glu</u> <u>Ala</u> <u>Leu</u> <u>Phe</u> <u>Asp</u> <u>Ala</u> <u>Asn</u> <u>Gln</u> Asn Thr	60	
181	AAA ACC GCT AAA ATT GAA ATC AAA GCC AGC	210	
61	Lys Thr Ala Lys Ile Glu Ile Lys Ala Ser	70	
211	CTC GAT GGT CTT GAA ATT GAT GTT CCC GGT	240	
71	Leu Asp Gly Leu Glu Ile Asp Val Pro Gly	80	
241	ATC GAT ACC AAT GCT TGC CAT TTT GTC AAA	270	
81	Ile Asp Thr Asn Ala Cys His Phe Val Lys	90	
271	TGT CCA TTG GTT AAA GGT CAA CAA TAT GAT	300	
91	Cys Pro Leu Val Lys Gly Gln Gln Tyr Asp	100	
301	ATC AAA TAT ACA TGG AAT GTG CCG AAA ATT	330	
101	Ile Lys Tyr Thr Trp Asn Val Pro Lys Ile	110	
331	GCA CCA AAA TCT GAA AAC GTT GTC GTT ACA	360	
111	Ala Pro Lys Ser Glu Asn Val Val Val Thr	120	
361	GTC AAA CTT ATC GGT GAT AAT GGT GTT TTG	390	
121	Val Lys Leu Ile Gly Asp Asn Gly Val Leu	130	
391	GCT TGC GCT ATT GCT ACC CAT GGT AAA ATC	420	
131	Ala Cys Ala Ile Ala Thr His Gly Lys Ile	140	
421	CGT GAT TAA AAA AAA ATA AAT AAG AAA ATT	450	
141	Arg Asp ***		
451	TTC ACC AAC ATC GAA CAA AAT TCA ATA ACC	480	
481	AAA ATT TGA ATC CAA AAA AAA AAA AAA	510	
511	ACC ATG G		
← Adaptor			

Fig. 1. Nucleotide and predicted amino acid sequence of the cDNA from pFL1. The amino acid residues underlined are identical to those of native *Der f* II.

*** is stop codon.

quences from the cDNAs.

DISCUSSION

The two genes coded *Der f* II were isolated from the cDNA library of *D. farinae*. There were minor differences observed between the two genes, but both gene products reacted similarly with anti-*Der f* II antibody. Therefore, the minor differences in the amino acid sequence do not seem to be very important in determining their antigenicity. Furthermore, it seems that *Der f* II protein does not

Adaptor			
1	GGT ACC ATG GTT TCA TTG TTG GTA GCA GCC	30	
1	Gly Thr Met Val Ser Leu Leu Val Ala Ala	10	
31	GTT GTT GCC GAT CAA GTC GAT GTT AAA GAT	60	
11	Val Val Ala <u>Asp</u> <u>Gln</u> Val <u>Asp</u> Val <u>Lys</u> <u>Asp</u>	20	
61	TGT GCC AAC AAT GAA ATC AAA AAA GTA ATG	90	
21	(Cys) <u>Ala</u> <u>Asn</u> <u>Asn</u> <u>Glu</u> <u>Ile</u> <u>Lys</u> <u>Lys</u> Val Met	30	
91	GTC GAT GGT TGC CAT GGT TCT GAT CCA TGC	120	
31	Val <u>Asp</u> <u>Gly</u> (Cys) <u>His</u> <u>Gly</u> Ser <u>Asp</u> Pro(Cys)	40	
121	ATC ATC CAT CGT GGT AAA CCA TTC ACT TTG	150	
41	<u>Ile</u> <u>Ile</u> <u>His</u> <u>Arg</u> <u>Gly</u> <u>Lys</u> Pro <u>Phe</u> Thr Leu	50	
151	GAA GCC TTA TTC GAT GCC AAC CAA AAC ACT	180	
51	<u>Glu</u> <u>Ala</u> <u>Leu</u> <u>Phe</u> <u>Asp</u> <u>Ala</u> <u>Asn</u> <u>Gln</u> Asn Thr	60	
181	AAA ACC GCT AAA ATT GAA ATC AAA GCC AGC	210	
61	Lys Thr Ala Lys Ile Glu Ile Lys Ala Ser	70	
211	CTC GAT GGT CTT GAA ATT GAT GTT CCC GGT	240	
71	Leu Asp Gly Leu Glu Ile Asp Val Pro Gly	80	
241	ATC GAT ACC AAT GCT TGC CAT TTT <u>ATG</u> AAA	270	
81	Ile Asp Thr Asn Ala Cys His Phe <u>Met</u> Lys	90	
271	TGT CCA TTG GTT AAA GGT CAA CAA TAT GAT	300	
91	Cys Pro Leu Val Lys Gly Gln Gln Tyr Asp	100	
301	<u>GCC</u> AAA TAT ACA TGG AAT GTG CCG AAA ATT	330	
101	<u>Ala</u> Lys Tyr Thr Trp Asn Val Pro Lys Ile	110	
331	GCA CCA AAA TCT GAA AAC GTT GTC GTT ACA	360	
111	Ala Pro Lys Ser Glu Asn Val Val Val Thr	120	
361	GTC AAA CTT <u>GTT</u> GGT GAT AAT GGT GTT TTG	390	
121	Val Lys Leu <u>Val</u> <u>Gly</u> Asp Asn Gly Val Leu	130	
391	GCT TGC GCT ATT GCT ACC CAC <u>GCT</u> AAA ATC	420	
131	Ala Cys Ala Ile Ala Thr His <u>Ala</u> Lys Ile	140	
421	CGT GAT TAA AAA AAA AAA ATA AAT ATG AAA	450	
141	Arg Asp ***		
451	ATT TTC ACC AAC ATC GAA CAA AAT TCA ATA	480	
481	ACC AAA ATT TGA ATC AAA AAA AAA AAA CCA	510	
511	TGC		
← Adaptor			

Fig. 2. Nucleotide and predicted amino acid sequence of the cDNA from pFL1. The amino acid residues underlined are identical to those of native *Der f* II.

*** is stop codon. The amino acid residues marked with boxes show the different position from pFL1.

consist of a single molecule but may be a mixture of different molecules. It will be important to clarify this point in the future.

Since it has already been reported that *Der f* II has similar antigenicity to *Der p* II¹⁰⁾, it may be proposed that both allergens have common regions. Indeed, Heymann *et al.* clarified the amino acid sequences of *Der p* II and *Der f* II from the N-terminals to the 35th amino acid residue. Both the partial sequences were very similar each other¹¹⁾. The amino acid sequence of *Der f* II based on the cDNA sequence showed good agreement with their results. The comparison of the total

amino acid sequences of *Der p* II and *Der f* II is very interesting. Therefore, the cloning of *Der p* II gene may be necessary for the study.

Since it has become possible to produce *Der f* II allergen by the genes coded with *Der f* II, the technique may be used for the production of *Der f* II in large quantity. If the desensitization therapy as well as diagnosis by standardized and purified mite major allergen is available for various atopic diseases caused by house dust mites, it will be of great advantage to the clinicians as well as those working on basic research on allergies.

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ダニ主要アレルゲン *Der f* II をコードする cDNA の クローニングとその塩基配列

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ダニ (*Dermatophagoides farinae*) の主要アレルゲン *Der f* II をコードする遺伝子を単離することを目的として、ダニ虫体より mRNA を分離精製し、対応する cDNA ライブラリーを作製した。そのライブラリーから抗 *Der f* II 抗体と反応するクローンをコロニーイムノアッセイで検索し、1600株より2株を分離した。得られたクローンより cDNA 配列部分を切り出しその塩基配列を決定したところ、二つの cDNA はどちらも128個のアミノ酸をコードしており三つのアミノ酸残基を除いて両者は一致していた。また *Der f* II 蛋白を精製しそのアミノ酸配列を N 末端より45残基まで決定した。その配列を cDNA から想定されるアミノ酸配列と比較したところ完全に一致しており、cDNA のコードする蛋白はダニ主要アレルゲン *Der f* II であると結論した。
