

Sequence analysis and expression of a cDNA clone encoding a 98-kDa allergen in *Dermatophagoides farinae*

L.-C. TSAI*, Y.-C. SUN†, P.-L. CHAO*, H.-P. NG*§, M.-W. HUNG*, K.-H. HSIEH†, S.-H. LIAW‡ and K.-Y. CHUA†§

*Department of Medical Research, Veterans General Hospital, Taipei, Graduate Institutes of †Immunology and ‡Molecular Medicine, College of Medicine, National Taiwan University, Taiwan and §Department of Paediatrics, Faculty of Medicine, National University of Singapore, Singapore

Summary

Background The important dust mite allergens identified to date are of molecular weights ranging from 14 to 60 kDa. Our previous protein study indicated that the 98-kDa native paramyosin in *Dermatophagoides farinae* mite showed IgE reactivity with 82% of the mite-sensitive asthmatic patients suggesting that it is a novel major mite allergen. This study described the isolation and characterization of the cDNA clone encoding the 98-kDa mite allergen.

Methods A *Dermatophagoides farinae* cDNA library was constructed in lambda ZAPII vector and the library was immunoscreened with a monoclonal antibody 642. The cDNA insert was sub-cloned into M13 sequencing vector for single-stranded sequencing. The whole cDNA insert was expressed in pGEX-2T *Escherichia coli* expression system as a fusion protein with GST. The allergenicity of the recombinant peptides was tested by skin tests and IgE immunoassay. The IgE and IgG immunoassays were performed with sera from 20 mite-allergic patients.

Results The cDNA clone Df642 was 2134 bp long, coding for a polypeptide of 711 amino acid residues. Protein sequence analysis and alignment confirmed that the deduced polypeptide is a mite paramyosin which is truncated slightly at the N- and C-terminuses. *In vivo* skin tests and *in vitro* IgE-binding study showed that 62% (13/21) and 50% (10/20) of the mite-sensitive asthmatic patients reacted positively with the recombinant *Dermatophagoides farinae* paramyosin, respectively.

Conclusion The study indicated that 98-kDa mite paramyosin is an important allergen.

Keywords: cDNA clone, *Dermatophagoides farinae*, mite allergen, paramyosin

Clinical and Experimental Allergy, Vol. 29, pp. 1606–1613. Submitted 26 October 1998; revised 13 January 1999; accepted 27 January 1999.

Introduction

Allergens derived from house dust mites have been regarded as an important source of indoor allergens [1]. Sensitization by mite allergens in genetically predisposed atopic individuals triggers a cascade of allergic reactions resulting in production of allergen-specific IgE [2]. Epidemiological studies revealed that there is increasing evidence that house dust mite allergy is a world-wide health problem

affecting about 10–20% of the world population [1]. The two most important clinical mite species are *Dermatophagoides pteronyssinus* (Dp) and *Dermatophagoides farinae* (Df), the allergens from these closely related species showed high degree of IgE-cross reactivity. *In vitro* IgE immunoassays revealed that groups I and II allergens reacted to specific IgE present in 80–90% of mite-allergic sera [3,4]. Whereas the rest of the mite allergens showed up to 40–50% IgE reactivity *in vitro* [5–7]. The mite allergens identified to date are of relatively low molecular weight ranging from 14 to 60 kDa, and more than 50% of them are enzymes [8]. In this study we report the molecular cloning

Correspondence: K.-Y. Chua, Department of Paediatrics, Faculty of Medicine, National University of Singapore, Lower Kent Ridge Road, Singapore 119074.

and sequence analysis as well as over-expression of a novel mite allergen from Df. As described in our previous study, the molecular weight of this newly characterized novel mite allergen is approximately 98 kDa [9]. The amino acid sequence data of the internal peptides derived from the native protein showed significant sequence homology with paramyosins from the invertebrates. The amino acid sequence deduced from the cDNA clone described in this report confirmed that the 98-kDa mite allergen is indeed a mite paramyosin. The *in vivo* skin test and *in vitro* IgE binding showed that 62 and 50% of mite-allergic patients reacted with the recombinant Df642, respectively. This is the first report describing the identification and characterization of a cDNA clone encoding a high molecular weight mite allergen, which is probably a mite structural protein.

Materials and methods

Sera

Human allergic sera were obtained from 20 asthmatic children, 3–14 years of age who attended the Paediatrics Allergy Clinic of the National Taiwan University Hospital, Taipei, Taiwan.

Construction of cDNA library

Breeding pairs of Df mites from house dust samples were collected from the domestic houses in Taipei, Taiwan. The mites were bred at 25 °C and 75% humidity in 15-cm Petri dishes containing yeast extract and fish powder as culture medium. Total RNA from one gram of live mites was isolated using RNazol™ B kit (Biotecx Laboratories, Inc. Houston, TX, USA) according to a modified method of Chirgwin *et al* [10]. The cDNA library was constructed by a standard protocol using lambda ZAPII expression vector (Stratagene, La Jolla, CA, USA). The lambda phage expression library was screened with a monoclonal antibody 642 (mAb642), which has been shown to react specifically to an allergenic 98-kDa protein from Df crude extract [9]. Approximately 2×10^5 phages were plated out with XL1-Blue *Escherichia coli* host on a 150-mm Petri dish containing Luria broth medium supplemented with 10 µg/mL of tetracycline. After 3.5 h of incubation at 42 °C, the plate was over-layed with a nitrocellulose filter previously saturated with 10 mmol/L isopropyl β-D-thiogalactopyranoside to induce antigen expression. The filter was then used for immunoscreening after blocking with 20% fetal calf serum in TRIS-buffered saline containing Tween-20 (TBST, 50 mmol/L TRIS pH 7.5, 150 mmol/L NaCl, 0.05% Tween-20). Primary antibody (mAb642) was used at 1:5000 dilution, the signal detection was performed using the combination of alkaline phosphatase-conjugated

secondary antibody (antimouse Ig, Sigma, St Louis, MO, USA). The TBST buffer was used throughout the immunoassay procedure. Thirty-eight recombinant phages reacted positively with mAb642, a selected panel of these clones was purified to homogeneity for further analysis. One of these cDNA clones was designated as Df642.

Sequence analysis of Df642 cDNA clone

The phagemid containing the cDNA insert of Df642 clone was excised *in vivo* from the lambda DNA with the help of a f1 helper phage according to a protocol described in the instruction manual of lambda ZAPII expression vector kit (Stratagene). To facilitate DNA sequencing, single-stranded templates were prepared by using the M13 phage [11]. DNA sequencing was performed with dideoxynucleotide chain termination method [12] using Sequenase version 2.0 (US Biochemical Corp., Cleveland, OH, USA).

Immunoassay for human IgE and IgG

The dot-blot immunoassay for IgE and IgG was performed by a protocol previously described [13]. Two micrograms of each purified protein was applied onto nitrocellulose membrane in duplicate. The dot-blot was blocked with phosphate-buffered saline (PBS) containing 5% skim milk and 0.1% Tween-20. Allergic sera were diluted 1:1 in PBS and incubated with the dot-blot for 12–16 h at 4 °C. The incubation with the secondary antibodies was performed with 1 in 1000 dilution of biotinylated mouse antihuman IgE (PharMingen, San Diego, CA, USA) or biotinylated mouse antihuman IgG (PharMingen) for 1 h, followed by 1 h incubation with extravidin peroxidase (Sigma). The signal of the reaction was developed using enhanced chemiluminescent detection reagent (Amersham, Amersham, UK) and autoradiography. Extensive washing of the dot-blot was performed with PBS containing 0.1% Tween-20 following each treatment step throughout the immunoassay protocol. Western blots were prepared by separating protein samples on a sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose membranes by protocols previously described [13].

Protein sequence analysis

Homologues to the deduced amino acid sequence of Df642 were searched through protein and gene databases by using the Blast program. The sequence analysis was performed by the GCG sequence analysis package. The prediction of coiled-coil conformation was performed using a MultiCoil algorithm [14]. The entire 711 amino acid residues were scanned with a window length of 28 residues. The protein sequence alignment was performed

by CLUSTAL W version 1.6 multiple sequence alignment software [15].

Expression of Df642 cDNA clone in E. coli

A BamHI fragment containing the entire Df642 cDNA sequence was produced by polymerase chain reaction (PCR) amplification. The PCR product was sub-cloned into the expression vector pGEX-2T and expressed as glutathione-S-transferase (GST) fusion proteins in *E. coli* [16]. Production and purification of the recombinant protein was prepared by protocol previously described [13]. Briefly, bulk culture of recombinant was grown in Luria broth containing 100 µg/mL of ampicillin to log phase and then induced with 1 mmol/L of isopropyl β-D-thiogalactopyranoside. After cell harvesting, cell lysate was prepared by sonication and protein purification was performed by using glutathione affinity chromatography.

Skin tests

Skin tests were performed according to previously described method [5]. Briefly, 0.02 mL of purified Df642-GST fusion protein at a concentration of 1 µg/mL was injected intradermally and reaction was scored at 15 min. GST fusion partner was used as a control. A weal size of greater than 6 mm was scored as a positive reaction. In cases in which reactions to the control were noted, these were subtracted from the size of the test reactions for subsequent evaluation. Subjects recruited for skin testing were children aged 10–14 years with moderate to severe asthma who attended the Paediatrics Allergy Clinics of National Taiwan University Hospital, Taiwan. This study was approved by the local ethics committee.

Results

Isolation of cDNA clone and cDNA sequence analysis

Thirty-eight positive plaques were obtained after screening approximately 5×10^4 plaque-forming units from the Df cDNA library with the monoclonal antibody 642. One of these plaques (Df642) was purified to homogeneity and the cDNA insert was sequenced with dideoxynucleotide sequencing method. The bluescript vector containing the insert was excised from the lambda phage and the purified recombinant plasmid was used for DNA sequencing. However, certain AT-rich regions of the cDNA insert did not permit the use of the conventional double-stranded DNA sequencing method, single-stranded DNA templates were used for sequencing. The cDNA clone was 2134 bp in length and contained 62% AT and 38% GC. Six phases translation

analysis revealed the polypeptide deduced from this cDNA clone showed a frame-shift translation at nucleotide position 57 (Fig. 1). The shift from phase I to phase II translation resulted in the conversion of a termination codon (TGA at nucleotide positions 58–60) to an initiation codon (ATG at nucleotide positions 57–59). The occurrence of this (–1) frame shift translation is probably due to the presence of the unique tetranucleotides at nucleotide positions 57–60, resulting in the formation of the overlapping ATG and TGA codons. The ATGA tetranucleotide overlapping sequence is preceded with a purine-rich region separated by four nucleotides upstream of ATG codon. The frame shift resulted in stop codon readthrough. The exact mechanism remains unclear at this stage, however, the presence of a 3' pseudoknot structure that resembles the 3' stimulator for –1 frame shift. This unique overlapping initiation and termination codons resulting in frame-shift translation have been described in tryptophan operon of *E. coli* and lambda bacteriophage DNA [17,18]. The deduced polypeptide consisted of 711 amino acid residues with an approximate molecular weight of 83 kDa and was named as Df642. Beside the ATG codon located at nucleotide positions 57–59 where the frame shift occurred, no further upstream N-terminal ATG codon was found, and there was no further termination codon found at the C-terminal of the sequence. The absence of both the stop codon and the typical polyadenylated site at the C-terminal of the cDNA insert suggests that Df642 is not a full-length cDNA clone. Amino acid sequences obtained from tryptic peptides derived the native counterpart of Df642 matched well with that of the deduced peptides except the residues at positions 844 (L to A), 1256 (R to A) and 1348 (E to G). We have confirmed that these discrepancies were not due to sequencing errors, and therefore probably reflect a significant degree of sequence polymorphism, which is a common feature of mite allergens. It has been demonstrated by western analysis of a two-dimensional gel that native counterpart of Df642 exist in multiple isoforms [9] and similar observation has been reported for a number of mite allergens [19,20].

Df642 encoded a protein that shared high sequence homology with paramyosin

The nucleotide and deduced amino acid sequences were compared with those in the Swiss-Prot and GeneBank databases using FASTA, and the sequence analysis and alignments were performed using the GCG and CLUSTAL W packages. The homology search revealed that this high molecular Df allergen, Df642, showed high degree of sequence identity with paramyosin from invertebrates. Sequence alignments showed that the 711 partial amino acid residues of Df642 protein shared 60% identity and

1	GGG	AGC	CTT	GGT	GAA	CGT	TTA	GAA	GAG	GCC	GAA	GAT	CAA	GCG	AAA	GTG	TTA	51
(1)	G	S	L	G	E	R	L	E	E	A	E	D	Q	A	K	V	L	(17)
52	CCG	<u>AA</u>	<u>ATG</u>	AAC	AAA	AAA	AGA	GAT	TCC	GAA	TTG	GCA	AAA	TTG	AGA	AAA	TTG	101
(18)	P	<u>K</u>	<u>M</u>	N	K	K	R	D	S	E	L	A	K	L	R	K	L	(34)
102	TTG	GAA	GAT	GTT	CAC	ATT	GAA	TCG	GAA	GAA	ACG	GCC	CAT	CAT	TTG	CGA	CAG	152
(35)	L	E	D	V	H	I	E	S	E	E	T	A	H	H	L	R	Q	(51)
153	AAA	CAT	CAG	GCT	GCC	ATC	CAG	GAA	ATG	CAA	GAT	CAA	CTT	GAC	CAA	TTG	CAA	203
(52)	K	H	Q	A	A	I	Q	E	M	Q	D	Q	L	D	Q	L	Q	(68)
204	AAA	GCG	AAA	AAT	AAA	TCG	GAT	AAA	GAG	AAG	CAA	AAA	TTT	CAG	GCT	GAA	GTT	254
(69)	K	A	K	N*	K*	S*	D	K	E	K	Q	K	F	Q	A	E	V	(85)
255	TTT	GAA	TTA	TTA	GCT	CAA	TTG	GAA	ACG	GCC	AAT	AAG	GAA	AAA	TTA	ACG	GCA	305
(86)	F	E	L	L	A	Q	L	E	T	A	N	K	E	K	L	T	A	(102)
306	TTG	AAA	AAT	GTA	GAA	AAA	TTG	GAA	TAT	ACC	GTA	CAT	GAA	TTG	AAT	ATC	AAA	356
(103)	L	K	N	V	E	K	L	E	Y	T	V	H	E	L	N	I	K	(119)
357	ATC	GAA	GAA	ATC	AAC	CGT	ACG	GTC	ATT	GAA	TTA	ACA	TCA	CAT	AAA	CAA	CGT	407
(120)	I	E	E	I	N*	R*	T*	V	I	E	L	T	S	H	K	<u>Q</u>	<u>R</u>	(136)
408	TTA	AGT	CAG	GAG	AAT	ACC	GAA	TTG	ATC	AAA	GAA	GTT	CAC	GAA	GTT	AAA	TTA	458
(137)	<u>L</u>	<u>S</u>	<u>Q</u>	<u>E</u>	<u>N</u>	<u>T</u>	<u>E</u>	<u>L</u>	<u>I</u>	<u>K</u>	<u>E</u>	<u>V</u>	<u>H</u>	<u>E</u>	<u>V</u>	<u>K</u>	<u>L</u>	(153)
459	CAA	TTG	GAC	AAT	GCA	AAC	CAT	TTG	AAG	ACA	CAG	ATT	GCC	CAA	CAA	TTA	GAA	509
(154)	<u>Q</u>	<u>L</u>	<u>D</u>	<u>N</u>	<u>A</u>	<u>N</u>	<u>H</u>	<u>L</u>	<u>K</u>	<u>T</u>	<u>Q</u>	<u>I</u>	<u>A</u>	<u>Q</u>	<u>Q</u>	<u>L</u>	<u>E</u>	(170)
510	GAC	ACT	CGA	CAT	CGT	TTG	GAA	GAA	GAG	GAA	CGA	AAA	CGT	GCC	AGT	CTC	GAA	560
(171)	D	T	R	H	R	L	E	E	E	E	R	K	A	<u>A</u>	<u>S</u>	<u>L</u>	<u>E</u>	(187)
561	AAT	CAT	GCC	CAT	ACA	TTG	GAA	GTG	GAA	TTA	GAA	TCA	TTG	AAA	GTA	CAA	TTG	611
(188)	<u>N</u>	<u>H</u>	<u>A</u>	<u>H</u>	<u>T</u>	<u>L</u>	<u>E</u>	<u>V</u>	<u>E</u>	<u>L</u>	<u>E</u>	<u>S</u>	<u>L</u>	<u>K</u>	<u>V</u>	<u>Q</u>	<u>L</u>	(204)
612	GAC	GAA	GAA	TCC	GAG	GCT	CGT	CTT	GAG	CTT	GAA	CGT	CAA	TTG	ACC	AAA	GCC	662
(205)	D	E	E	S	E	A	R	L	E	L	E	R	Q	L	T	K	A	(221)
663	AAT	GGC	GAT	GCT	GCA	TCA	TGG	AAG	TCC	AAA	TAC	GAA	GCT	GAA	TTG	CAA	GCA	713
(222)	N	G	D	A	A	S	W	K	S	K	Y	E	A	E	L	Q	A	(238)
714	CAT	GAA	GAT	GAA	GTT	GAA	GAA	CTT	CGT	CGT	AAA	ATG	GCT	CAA	AAG	ATT	TCG	764
(239)	H	A	D	E	V	E	E	L	R	R	K	M	A	Q	K	I	S	(255)
765	GAA	TAC	GAA	GAA	CAA	TTG	GAA	GCC	TTA	TTG	AAT	AAA	TGC	AGT	TCA	TTG	GAG	815
(256)	E	Y	E	E	Q	L	E	A	<u>L</u>	<u>L</u>	<u>N</u>	<u>K</u>	<u>C</u>	<u>S</u>	<u>S</u>	<u>L</u>	<u>E</u>	(272)
816	AAA	CAA	AAA	TCT	CGA	CTT	CAA	AGC	GAA	GTT	GAA	GTT	TTG	ATT	ATG	GAT	CTT	866
(273)	<u>K</u>	<u>Q</u>	<u>K</u>	<u>S</u>	<u>R</u>	<u>L</u>	<u>Q</u>	<u>S</u>	<u>E</u>	<u>V</u>	<u>E</u>	<u>V</u>	<u>L</u>	<u>I</u>	<u>M</u>	<u>D</u>	<u>L</u>	(289)
867	GAA	AAA	GCG	ACA	CGA	CAT	GCA	CAA	CAA	TTA	GAA	AAA	CGT	GTT	GCT	CAA	TTG	917
(290)	E	K	<u>A</u>	<u>T</u>	<u>R</u>	<u>H</u>	<u>A</u>	<u>Q</u>	<u>Q</u>	<u>L</u>	<u>E</u>	<u>K</u>	<u>R</u>	<u>V</u>	<u>A</u>	<u>Q</u>	<u>L</u>	(306)
918	GAA	AAG	ATT	AAT	CTT	GAT	TTG	AAG	AAT	AAA	TTG	GAA	GAG	GTT	ACC	ATG	TTG	968
(307)	<u>E</u>	<u>K</u>	I	N	L	D	L	K	N	K	L	E	E	V	T	M	L	(323)
969	ATG	GAA	CAA	GCA	CAG	AAA	GAA	CTT	CGA	GTC	AAG	ATT	GCT	GAA	TTA	CAG	AAA	1019
(324)	M	E	Q	A	Q	K	E	L	R	V	K	I	A	E	L	Q	K	(340)
1020	TTG	CAA	CAT	GAA	TAT	GAA	AAA	TTA	CGT	GAT	CAA	CGT	CAT	CAA	TTG	GCA	CGT	1070
(341)	<u>L</u>	<u>Q</u>	<u>H</u>	<u>E</u>	<u>Y</u>	<u>E</u>	<u>K</u>	<u>L</u>	<u>R</u>	<u>D</u>	<u>Q</u>	<u>R</u>	<u>D</u>	<u>Q</u>	<u>L</u>	<u>A</u>	<u>R</u>	(357)
1071	GAA	AAC	AAG	AAA	CTT	ACA	GAC	GAT	CTT	GCC	GAA	GCT	AAA	TCA	CAA	TTG	AAC	1121
(358)	<u>E</u>	<u>N</u>	<u>K</u>	<u>K</u>	<u>L</u>	<u>T</u>	<u>D</u>	<u>D</u>	<u>L</u>	<u>A</u>	<u>E</u>	<u>A</u>	<u>K</u>	<u>S</u>	<u>Q</u>	<u>L</u>	<u>N</u>	(374)
1122	GAT	GCT	CAC	CGT	AGA	ATC	CAT	GAA	CAA	GAA	ATT	GAA	ATC	AAA	CGA	TTA	GAG	1172
(375)	D	A	H	R	R	I	H	E	Q	E	I	E	I	K	R	L	E	(391)
1173	AAT	GAA	CGT	GAT	GAA	TTA	TCG	GCT	GCC	TAT	AAA	GAA	GCA	GAA	ACA	TTG	AGA	1223
(392)	N	E	R	D	E	L	S	A	A	Y	K	E	A	E	T	L	R	(408)
1224	AAA	CAA	GAA	GAG	GCC	AAA	AAT	CAA	CGA	TTG	ATT	GCC	GAA	TTG	GCA	CAG	GTA	1274
(409)	K	Q	E	E	A	K	N	<u>Q</u>	<u>R</u>	<u>L</u>	<u>I</u>	<u>A</u>	<u>E</u>	<u>L</u>	<u>A</u>	<u>Q</u>	<u>V</u>	(425)
1275	CGA	CAT	GAT	TAT	GAA	AAA	CGT	TTG	GCA	CAA	AAA	GAT	GAA	GAA	ATT	GAA	GCA	1325
(426)	<u>R</u>	H	D	Y	E	K	R	L	A	Q	K	D	<u>E</u>	<u>E</u>	<u>I</u>	<u>E</u>	<u>A</u>	(442)
1326	TTG	CGC	AAA	CAA	TAT	CAA	ATT	GAA	ATT	GAA	CAA	CTT	AAC	ATG	CGA	TTG	GCC	1376
(443)	<u>L</u>	R	K	Q	Y	Q	I	E	I	E	Q	L	N	M	R	L	A	(459)
1377	GAG	GCT	GAA	GCT	AAA	CTC	AAG	ACC	GAA	ATT	GCA	CGA	TTG	AAG	AAA	AAA	TAT	1427
(460)	E	A	E	A	K	L	K	T	E	I	A	R	L	K	K	K	Y	(476)

1428	CAG	GCA	CAG	ATT	ACC	GAA	TTG	GAA	TTG	TCA	TTG	GAT	GCA	GCC	AAT	AAG	GCT	1478
(477)	Q	A	Q	I	T	E	L	E	L	S	L	D	A	A	N	K	<u>A</u>	(493)
1479	AAT	ATC	GAT	TTG	CAA	AAG	ACG	ATC	AAA	AAA	CAA	GCC	CTT	CAA	ATT	ACG	GAG	1529
(494)	<u>N</u>	<u>I</u>	<u>D</u>	<u>L</u>	<u>Q</u>	<u>K</u>	T	I	K	K	Q	A	L	Q	I	T	E	(510)
1530	CTC	CAA	GCA	CAT	TAT	GAT	GAA	GTT	CAT	CGT	CAA	TTG	CAA	CAA	GCA	GTG	GAT	1580
(511)	L	Q	A	H	Y	D	E	V	H	R	Q	L	Q	Q	A	V	D	(527)
1581	CAA	TTG	GGT	GTT	ACA	CAA	CGA	CGA	TGC	CAA	GCA	TTG	CAA	GCC	GAA	TTG	GAA	1631
(528)	Q	L	G	V	T	Q	R	R	C	Q	A	L	Q	A	E	L	E	(544)
1632	GAG	ATG	CGT	ATT	GCA	TTG	GAA	CAG	GCT	AAT	CGT	GCT	AAA	CGA	CAA	GCC	GAA	1682
(545)	E	M	R	I	A	L	E	Q	A	N	R	A	K	R	Q	A	E	(561)
1693	CAA	TTG	CAT	GAA	GAA	GCT	GTT	GTA	CGT	GTT	AAC	GAA	CTT	ACC	ACA	ATT	AAC	1733
(562)	Q	L	H	E	E	A	V	V	R	V	N	E	L	T	T	I	N	(578)
1734	GTC	AAT	TTG	GCA	TCG	GCT	AAA	AGT	AAA	TTG	GAA	TCA	GAA	TTC	TCT	GCA	CTT	1784
(579)	V	N	L	A	S	A	K	S	K	L	E	S	E	F	S	A	L	(595)
1785	CAA	GCT	GAT	TAC	GAT	GAA	GTA	CAT	AAA	GAA	CTT	AGA	ATT	TCT	GAT	GAA	CGA	1835
(596)	Q	A	D	Y	D	E	V	H	K	<u>E</u>	<u>L</u>	<u>R</u>	<u>I</u>	<u>S</u>	<u>D</u>	<u>E</u>	<u>R</u>	(612)
1836	GTA	CAG	AAA	CTT	ACA	ATT	GAA	CTC	AAA	TCT	ACT	AAA	GAT	TTG	TTG	ATC	GAA	1886
(613)	<u>V</u>	<u>Q</u>	<u>K</u>	L	T	I	E	L	K	S	T	K	D	L	L	I	E	(629)
1887	GAA	CAA	GAA	CGA	TTG	GTT	AAA	TTG	GAA	ACG	GTG	AAA	AAA	TCA	TTG	GAA	CAA	1937
(630)	E	Q	E	R	L	V	K	L	E	T	V	K	K	S	L	E	Q	(646)
1938	GAG	GTA	CGA	ACA	TTG	CAT	GTC	CGT	ATT	GAA	GAG	GTC	GAA	GCC	AAT	GCA	TTG	1988
(647)	E	V	R	T	L	H	V	R	I	E	E	V	E	A	N	A	L	(663)
1989	GCC	GGT	GGT	AAA	CGT	GTC	ATT	GCC	AAA	TTG	GAA	AGC	CGA	ATT	CGT	GAT	GTT	2039
(664)	A	G	G	K	R	V	I	A	K	L	E	S	R	I	R	D	V	(680)
2040	GAA	ATT	GAA	GTT	GAA	GAA	GAA	CGA	CGA	CGA	CAT	GCC	GAA	ACG	GAC	AAA	ATG	2090
(681)	E	I	E	V	E	E	E	R	R	R	H	A	E	T	D	K	M	(697)
2091	TTA	CGT	AAA	AAG	GAT	CAT	CGT	GTC	AAG	GAA	TTG	TTG	TTG	CAA	AA			2134
(698)	L	R	K	K	D	H	R	V	K	E	L	L	L	Q				(711)

Fig. 1. Nucleotide and deduced amino acid sequence of Df642. The numbers labelled the positions of the nucleotides and amino acid residues (in parentheses). The predicted leucine zipper motifs are shown in bold italicized letters. Amino acid sequences determined by Edman degradation are underlined. The two predicted *N*-linked glycosylation sites are indicated by asterisks.

76% homology with the paramyosin of *Drosophila melanogaster*. It also shared 50% identity, 70% homology with paramyosins from *Caenorhabditis elegans*, *Onchocerca volvulus*, *Brugia malayi*, *Dirofilaria immitis*, and 34% identity, 55% homology to those of *Schistosoma japonicum*, *S. mansoni*, *Taenia solium*, and *Echinococcus granulosus*. There are two putative *N*-glycosylation sites present at residues number 72–74 and 124–126, they are probably functional because the presence of high mannose *N*-glycan chains has been detected in native protein [9]. As indicated in Fig. 1, several relatively long leucine zipper motifs were found, these motifs may not be the binding site of the DNA-binding protein, however, they play an important structural role in the folding of the protein instead.

Antibody binding capacity of recombinant Df642

A BamHI DNA fragment derived from Df642 cDNA insert was produced by PCR amplification and sub-cloned into pGEX-2T expression vector. The resulting recombinant

Df642 protein (rDf642) expressed as a fusion protein with glutathione-S-transferase (GST) was estimated as about 120 kDa in size on a SDS-PAGE gel. This affinity purified recombinant fusion protein reacted strongly with human IgE from mite-allergic sera (Fig. 2, lane 3) as well as with the mAb642 (Fig. 2, lane 6). The recombinant protein, like its native counterpart, appeared to be very susceptible to degradation giving rise to a number of smaller fragments ranging from 45 to 120 kDa in size, and the derived fragments were reactive to human IgE and mAb642 monoclonal antibody. The reactivity of human IgE and IgG to the recombinant Df642 was also studied by immunodot assay with a panel of 20 allergic sera. Figure 3 showed the *in vitro* IgE and IgG binding results for a representative panel of three allergic sera and one nonatopic control serum. The data of the IgG and IgE binding were summarized in Table 1. Overall, 55% (11 out of 20 allergic sera) showed positive IgE and IgG reactivity to the rDf642. The GST and recombinant Der p 2 (rDer p 2) proteins were included as negative and positive controls, respectively.

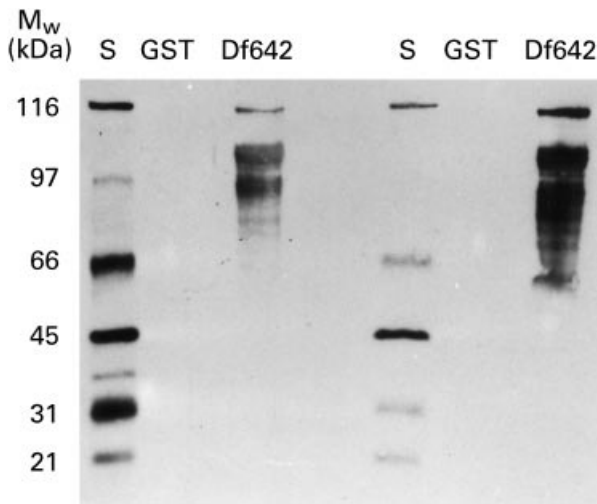


Fig. 2. Western blot analysis of recombinant Df642. The recombinant Df642 was produced as a fusion with glutathione-S-transferase (GST). Human IgE immunoassay was performed with a pooled sera from asthmatic patients (lane 3). The IgG immunoassay was performed with mAb642 (lane 6). Lanes 1 and 4: molecular weight markers; lanes 2 and 5: GST protein controls; lanes 3 and 6: GST-Df642 recombinant protein.

Skin tests

The rDf642 was also skin-tested on 21 mite-sensitive subjects. These were children aged 8–15 years with moderate to severe asthma who attended the Paediatrics Allergy Clinics of National Taiwan University Hospital, Taiwan. Table 2 showed the skin tests results from these 21 patients. Fifteen out of 21 showed reactions to rDf642, nine cases also showed reactions to GST control protein, these were subtracted from the size of the test reactions for subsequent evaluation. The final analysis showed that approximately 62% of the patients reacted positively with rDf642.

Discussion

The native Df642 has been isolated from Df mite extract in our laboratory using monoclonal antibody affinity chromatography. The native Df642 is a glycoprotein that is approximately 98 kDa in size. It has been shown that 82% (55 out of 67) of the mite skin-test positive sera obtained from asthmatic patients showed IgE reactivity to native Df642. This frequency of IgE reactivity is similar to that of the major group 1 and 2 mite allergens, and therefore suggesting Df642 is an important major mite allergen [9]. The clinical importance of this high molecular weight allergen in allergic asthma has been suggested by several studies [21,22], our study using the native Df642 confirmed

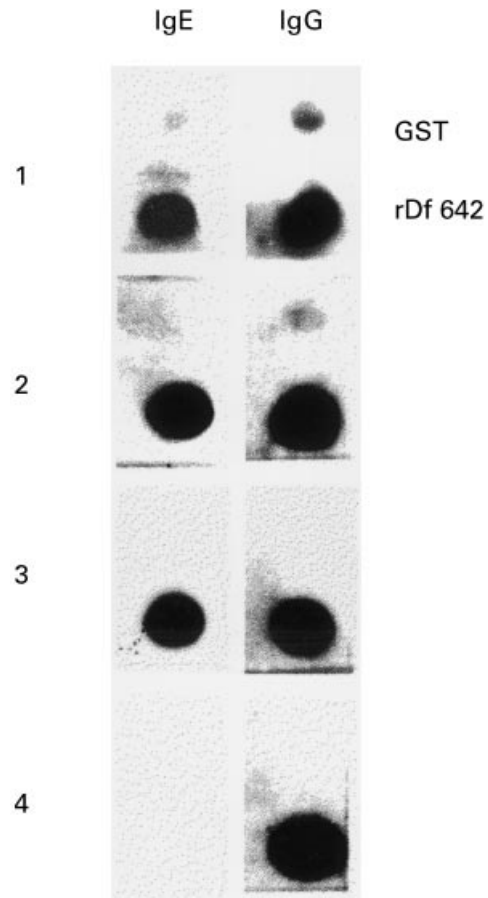


Fig. 3. IgE and IgG dot-blot immunoassay. Panels 1–3 indicate IgE and IgG reactivity of three selected allergic sera to recombinant GST-Df642 and GST control proteins. Panel 4 shows the IgE and IgG reactivity of a nonatopic serum to GST-Df642 and GST proteins.

the clinical importance of such a high molecular weight allergen. The present study reports for the first time the cDNA sequence coding for this novel 98-kDa molecular weight allergen. The cDNA clone described in this study codes for a 711-residue polypeptide of approximately 83 kDa. The amino acid sequence alignment showed that the deduced polypeptide is a paramyosin and it is truncated at both N- and C-terminuses. Despite the truncation the rDf642 protein reacted positively with 62% (13/21) of the mite-sensitive patients by skin-tests. The *in vitro* IgE binding assay showed that 50% (10/20) of the mite-sensitive sera reacted positively with the rDf642. However, our previous study showed that the human IgE binding frequency with the native Df642 was as high as 82%. The discrepancy in the results is probably due to the fact that the rDf642 is truncated at both N- and C-terminuses. Given the fact the Df642 is highly susceptible to degradation, and the

Table 1. Antibody binding capacity of GST-Df642 recombinant protein. Positive and negative bindings are shown by (+) and (–), respectively. GST protein served as a negative control.

Serum no.	Antigen					
	rDf 642		GST		rDer p 2	
	IgE	IgG	IgE	IgG	IgE	IgG
Nonatopic control	–	+	–	–	–	+
1	+	+	–	–	+	+
2	–	–	–	–	+	+
3	–	–	–	–	–	+
4	–	–	–	–	+	+
5	–	–	–	–	+	+
6	–	–	–	–	–	+
7	–	–	–	–	+	+
8	–	–	–	+	+	+
9	+	+	–	–	+	+
10	+	+	–	–	+	+
11	+	+	–	–	+	+
12	–	+	–	–	+	+
13	+	+	–	–	+	+
14	+	+	–	–	+	+
15	+	+	–	–	+	+
16	+	+	–	–	+	+
17	+	+	–	–	+	+
18	+	+	–	+	+	+
19	–	–	–	–	+	+
20	–	–	–	–	+	+
No. of positive reactions	10	11	0	2	18	20

degraded products were still reactive to human IgE, therefore it is conceivable that the mite-sensitive subjects were sensitized by the peptides derived from the whole molecule.

Paramyosin has been identified as a prominent immunogen in parasites and as a most promising vaccine candidate for parasites such as filariasis [23] and schistosome infection [24]. For instance, *T. solium* (Cestoda) paramyosin is a prominent antigen (antigen B) in human cysticercosis, and may have a role as a modulator of the host immune response [25,26]. Our studies revealed that Df mite paramyosin is a major allergen. Although more than 50% of the mite allergens characterized so far are enzymes, the group 10 mite allergens have been identified as muscle-associated tropomyosins [27]. Like the tropomyosin, paramyosin is a protein associated with muscles. Both tropomyosin and paramyosin composed of two identical coiled-coil alpha-helices. Unlike tropomyosin, paramyosin is only found in invertebrates and the distribution and function(s) of

Table 2. Skin tests reactions of 21 asthmatic patients to recombinant GST-Df642 and GST control proteins. A weal size greater than 6 mm (after subtracting the reaction to GST control from the test reaction) was considered as positive

Patient no.	Age (Years)	Sex	Antigen	
			GST-Df642 (Weal size/induration size in mm)	GST
1	14	M	65 × 60/25 × 20	–
2	10	F	50 × 40/15 × 10	–
3	10	M	10 × 8/4 × 3	–
4	10	M	7 × 8/4 × 4	–
5	15	M	45 × 45/10 × 8	25 × 20/5 × 5
6	13	M	35 × 35/8 × 8	15 × 15/5 × 3
7	12	M	55 × 40/10 × 10	9 × 9/4 × 4
8	10	M	20 × 20/5 × 8	–
9	12	M	35 × 30/10 × 10	–
10	7	M	10 × 8/4 × 5	8 × 8/4 × 4
11	8	F	15 × 15/5 × 5	9 × 9/4 × 4
12	12	M	8 × 8/4 × 4	4 × 4/2 × 2
13	13	M	35 × 35/10 × 10	9 × 9/4 × 4
14	11	M	45 × 30/10 × 10	10 × 10/5 × 5
15	8	F	35 × 30/8 × 10	8 × 6/3 × 3
16	9	M	–	–
17	12	M	–	–
18	7	M	–	–
19	12	M	–	–
20	10	M	–	–
21	10	F	–	–

paramyosin are quite diverse. Although paramyosin is usually known as a myofibrillar protein that is associated with the core structure of the thick filaments of invertebrate muscle, however, it has been localized in a non-filamentous membrane-bounded form in *Schistosoma teguments* and may be a secretory product of the tissue concerned [28,29]. It is conceivable that paramyosin can exist as a muscle structural protein as well as a secretory form. The two forms of paramyosins may perform different roles in the invertebrates. In this regard, it has been reported that paramyosins from invertebrates can inhibit the function of complement C1 in human serum [30]. This implies that paramyosin may play a role in the modulation of immune responses in the host. Paramyosin may also be involved in the regulation of catch contractions in molluscan smooth muscles. The phosphorylation of the paramyosin may be involved in such regulation [31]. It is unclear whether the paramyosins from the dust mites exist in more than one form. Given the fact that the dust mite tropomyosins have been identified as important allergens, it is therefore likely that the allergenic paramyosin from Df mite is a structural protein associated

with the mite body. However, the possibility that Df642 is a regulatory protein of the dust mite cannot be ruled out.

Acknowledgements

This study was supported by grants from the National Science Council (NSC81-0420-B075-561) and from the Veterans General Hospital, Taipei, Taiwan.

References

- Indoor Allergens and Asthma. Report of the 3rd International Workshop. Cuenca, Spain, June 30–July 2 1995.
- Ramagnani S. Regulation of the development of type 2 T-helper cells in allergy. *Current Opin Immunol* 1994; 6:838–46.
- Chua KY, Kehal PK, Thomas WR, Vaughan PR, Macreadie IG. High-frequency binding of IgE to the Der p allergen expressed in the yeast. *J Allergy Clin Immunol* 1992; 89:95–102.
- Chua KY, Doyle CR, Simpson RJ, Turker KJ, Stewart GA, Thomas WR. Isolation of cDNA coding for the major mite allergen Der p II by IgE plaque immunoassay. *Int Arch Allergy Appl Immunol* 1990; 91:118–23.
- Lin KL, Hsieh KH, Thomas WR, Chiang BL, Chua KY. Characterization of Der p V allergen, cDNA analysis, and IgE mediated reactivity to the recombinant protein. *J Allergy Clin Immunol* 1994; 94:989–96.
- Smith WA, Chua KY, Kuo MC, Rogers BL, Thomas WR. Cloning and sequencing of Dermatophagoides pteronyssinus group III allergen, Der p 3. *Clin Exp Allergy* 1994; 24:220–8.
- Shen HD, Chua KY, Hsieh KH, Thomas WR. Molecular cloning and immunological characterization of the group 7 allergens of house dust mites. In: Sehon A, Hayglass KT, Kraft D et al, eds. *New Horizons in Allergy Immunotherapy*. New York, NY, USA: Plenum Press, 1996, 241–2.
- Thomas WR. Mite allergen groups I–VII. A Catalogue *Enzymes Clin Exp Allergy* 1993; 23:350–3.
- Tsai LC, Chao PL, Shen HD et al. Isolation and characterization of novel 98 kDa Dermatophagoides farinae mite allergen. *J Allergy Clin Immunol* 1998; 102:295–303.
- Chirgwin JM, Przybyl AE, Macdonald RJ, Rutter WJ. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* 1979; 18:5294–9.
- Messing J. New M13 vectors for cloning. *Meth Enzymol* 1983; 101:20–78.
- Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain terminating inhibitors. *Proc Natl Acad Sci USA* 1977; 74:5463–7.
- Chua KY, Greene WK, Kehal PK, Thomas WR. IgE binding studies with large peptides expressed from Der p II cDNA constructs. *Clin Exp Allergy* 1991; 21:161–6.
- Wolf E, Kim PS, Berger B. MultiCoil. A program for predicting two- and three-stranded coiled coils. *Protein Sci* 1997; 6:1179–89.
- Thompson JD, Higgins DG, Gibson TJ, Clustal W. Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucl Acid Res* 1994; 22:4673–80.
- Smith DB, Johnson KS. Single-step purification of polypeptides expressed in Escherichia coli as fusion with glutathione S-transferase. *Gene* 1988; 67:31–40.
- Kroeger M, Hobom G. A chain of interlinked genes in the ninR region of bacteriophage, lambda. *Gene* 1982; 20:25–38.
- Oppenheim DS, Yanofsky C. Translational coupling during expression of the tryptophan operon of Escherichia coli. *Genetics* 1989; 95:785–95.
- Chua KY, Kehal PK, Thomas WR. Sequence polymorphism of cDNA clones encoding the mite allergen Der p I. *Int Arch Allergy Immunol* 1993; 101:364–8.
- Chua KY, Huang CH, Shen HD, Thomas WR. Analysis of sequence polymorphism of a major mite allergen Der p 2. *Clin Exp Allergy* 1996; 26:829–37.
- Lin KL, Wang SY, Hsieh KH. Analysis of house dust mite-specific IgE, IgG4, and IgG antibodies during immunotherapy in asthmatic children. *Ann Allergy* 1996; 67:63–9.
- Tang RB, Tsai LC, Hung MW, Hwang B, Wu KG. Detection of house dust mite allergens and immunoblot analysis in asthmatic children. *J Asthma* 1988; 25:83–8.
- Li BW, Chandrashekar R, Weil GJ. Vaccination with recombinant filarial paramyosin induces partial immunity to Brugia malayi infection in jirds. *J Immunol* 1993; 150:1881–5.
- Pearce EJ, James SL, Dalton J et al. Immunochemical characterization and purification of Sm-97, a Schistosoma mansoni antigen monospecifically recognized by antibodies from mice protectively immunized with a nonliving vaccine. *J Immunol* 1986; 11:3593–600.
- Laclette JP, Landa A, Arcos L, Willms K, Davis AE, Shoemaker CB. Paramyosin is the Schistosoma mansoni (Trematoda) homologue of antigen B from Taenia solium (Cestoda). *Mol Biochem Parasitol* 1991; 44:287–96.
- Landa A, Laclette JP, Shoemaker CB. cDNA cloning and recombinant expression of collagen-binding and complement inhibitor activity of Taenia solium paramyosin (AgB). *Mol Biochem Parasitol* 1993; 60:343–7.
- Aki T, Kodama T, Fujikawa A et al. Immunochemical characterization of recombinant and native tropomyosin as a new allergen from the dust mite, Dermatophagoides farinae. *J Allergy Clin Immunol* 1995; 96:74–83.
- Matsumoto Y, Perry G, Levine RJC, Blanton R, Mahmoud AAF, Aikawa W. Paramyosin and actin in Schistosomal Teguments. *Nature* 1998; 333:76–8.
- Gobert GN, Stenzel DJ, Jones MK, Allen DE, McManus DP. Schistosoma japonicum: immunolocalization of paramyosin during development. *Parasitology* 1997; 114:45–52.
- Laclette JP, Shoemaker CB, Richter D et al. Paramyosin inhibits complement C1. *J Immunol* 1992; 148:124–8.
- Castellani L, Cohen S. Myosin rod phosphorylation and the catch state of molluscan muscles. *Science* 1987; 235:334–7.