

Synthesis of Biologically Active Recombinant *Der f* II

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Abstract. A cDNA library corresponding to mite protein was screened employing anti-*Der f* II antibody. Two possible clones containing plasmids pFL1 and pFL11 were obtained. The two plasmids had insertions of about 500 basepairs. 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 *Dermatophagoides farinae* mite corresponded to the mite allergen *Der f* II. The recombinant *Der f* II was biologically active.

Five grams of live mites were rapidly frozen with liquid nitrogen. The purified mRNA from mites was prepared by guanidine isothiocyanate extraction and oligo(dT)-cellulose column chromatography. The cDNA library was prepared using a cDNA synthesizing system plus [1]. The cDNA library was expressed in *Escherichia coli* MC1061 employing an expression vector pUEX1 [2]. The clones were screened by colony immunoassay employing a rabbit anti-*Der f* II antibody and immunoscreening system. Two possible candidates were found among 1,600 clones. The cDNA insert was excised by BamHI. BamHI cDNA fragments were digested with various restriction endonucleases such as NcoI. The subcloning was carried out employing the plasmids pUC118 and pUC119 [3]. 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 (fig. 1, 2) by the method of Sanger [4]. The highly purified native *Der f* II protein [5] was subjected to amino acid sequence determination. Forty-five amino acid residues from the N terminus could be determined. This sequence was completely the same as the sequences deduced from the *Der f* II cDNAs and was very similar to that reported by Heymann et al. [6]. We concluded that the cDNAs prepared from live *Dermatophagoides farinae* mite corresponded to the mite allergen *Der f* II [1]. The recombinant *Der f* II protein was reactive with human antimites IgE antibody and able to induce an immediate-type skin reaction in a mite-sensitive atopic colleague (M.D.). The recombinant *Der f* II protein also released histamine from basophils obtained from mite-sensitive atopic individuals (fig. 3).

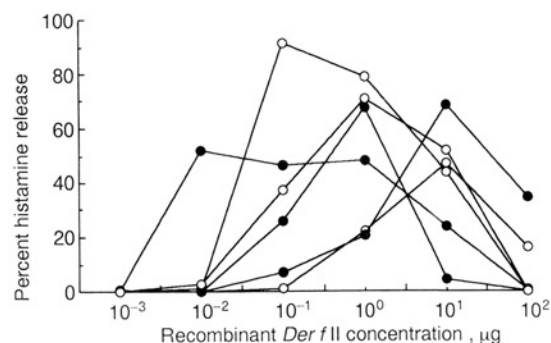
Adaptor			
1	GGT ACC ATG GTT TCA TTG TTG GTA GCA GCC	30	
1	Gly Thr Met Val Ser Leu Leu Val Ala Ala	10	
31	GTT GTT GCC GAT CAA GTC GAT GTT AAA GAT	60	
11	Val Val Ala <u>Asp</u> Gln Val <u>Asp</u> Val Lys <u>Asp</u>	20	
61	TGT GCC AAC AAT GAA ATC AAA AAA GTA ATG	90	
21	(Cys)Ala Asn Asn Glu Ile <u>Lys</u> Lys Val Met	30	
91	GTC GAT GGT TGC CAT GGT TCT GAT CCA TGC	120	
31	<u>Val Asp Gly</u> (Cys) <u>His</u> Gly Ser <u>Asp</u> Pro(Cys)	40	
121	ATC ATC CAT CGT GGT AAA CCA TTC ACT TTG	150	
41	<u>Ile</u> Ile His Arg Gly Lys Pro Phe Thr Leu	50	
151	GAA GCC TTA TTC GAT GCC AAC CAA AAC ACT	180	
51	<u>Glu</u> Ala Leu Phe <u>Asp</u> Ala Asn <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 ***	450	
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. *** = Stop codon.

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> Gln Val <u>Asp</u> Val Lys <u>Asp</u>	20	
61	TGT GCC AAC AAT GAA ATC AAA AAA GTA ATG	90	
21	(Cys)Ala Asn Asn Glu Ile <u>Lys</u> Lys Val Met	30	
91	GTC GAT GGT TGC CAT GGT TCT GAT CCA TGC	120	
31	<u>Val Asp Gly</u> (Cys) <u>His</u> Gly Ser <u>Asp</u> Pro(Cys)	40	
121	ATC ATC CAT CGT GGT AAA CCA TTC ACT TTG	150	
41	<u>Ile</u> Ile His Arg Gly Lys Pro Phe Thr Leu	50	
151	GAA GCC TTA TTC GAT GCC AAC CAA AAC ACT	180	
51	<u>Glu</u> Ala Leu Phe <u>Asp</u> Ala Asn <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> Gly 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 ***	450	
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. *** = Stop codon. The amino acid residues marked with boxes show the different positions from pFL1.

Fig. 3. Recombinant *Der f*II-mediated histamine release. Basophil-rich leukocyte fractions obtained from 6 mite-sensitive atopic patients were subjected to histamine release assay with recombinant *Der f*II.



References

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