

Original article

Immunologic characterization of the recombinant fire ant venom allergen Sol i 3

Individuals sensitized to fire ant stings show immunoglobulin (Ig)E antibodies against the venom protein Sol i 3. We determined the full-length complementary DNA (cDNA) sequence of this protein and expressed recombinant Sol i 3 in immunogenic form. The complete cDNA of Sol i 3 was obtained by reverse transcription polymerase chain reaction (RT-PCR) and PCR + 1 reactions using gene-specific oligonucleotides, and oligonucleotides designed from the amino acid sequence of this protein. The encoding cDNA is 705 bp in length corresponding to 235 amino acids. The first 22 amino acids are a leader sequence. The protein with an added C-terminal hexahistidine tag was expressed in insect cells using a baculovirus system. The recombinant protein was secreted into the supernatant and affinity purified with a cobalt chelating resin. The recombinant fire ant venom allergen Sol i 3 showed similar IgE binding activity to the native protein in radioallergosorbent test (RAST) and RAST inhibition assays. It was produced in both a glycosylated and an unglycosylated form. A three-dimensional reconstruction of Sol i 3 was compared with the experimentally determined structure of the related allergen Ves v 5. This model is supported by results of circular dichroism spectroscopy.

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Venoms of Hymenoptera, including vespids, honey bees, and fire ants are common causes of allergic reactions. Venom from the red imported fire ant, *Solenopsis invicta*, contains four important, potent allergens, which cause anaphylactic reactions in highly sensitive individuals (1). The amino acid sequences of three of these venom proteins, Sol i 2, 3, and 4 have been determined (2). Sol i 2, the most abundant of the venom proteins, has been cloned and expressed in its native form (3, 4). Sol i 3 with a molecular mass of 23 968 makes up 15–25% of the venom protein (1). Sol i 3 shows 44–50% identity with antigen 5's from vespids (5). Despite this similarity, no immunologic cross-reactivity between Sol i 3 and vespid antigen 5's has been observed with human immunoglobulin (Ig)E antibodies (6).

Whole body vaccines of imported fire ants have allergenic activity, and contain numerous non-venom proteins. In addition the quantities of allergen activity found in different vaccines are highly variable, and some commercial preparations are deficient in one or more

allergens (7). Pure venom shows significant increases in allergen specificity and activity (8). However, the venom contains piperidine alkaloids, which can cause an immediate wheal and flare reaction and which also cause the characteristic sterile pustule (9) of an imported fire ant sting.

Expression of allergens as recombinant proteins overcomes the problem of obtaining large quantities from the natural material, and the use of a hexahistidine tag simplifies purification. However, it is necessary to compare the recombinant proteins with the native proteins to ensure similar properties. Here, we report the complete complementary DNA (cDNA) sequence of the fire ant venom allergen Sol i 3, and the production and characterization of the recombinant protein in immunoreactive form.

Material and methods

Construction of oligonucleotide primers for PCR

A degenerate sense oligonucleotide primer (P1) was designed according to the known amino acid sequence. For the 3' RACE protocol oligo (dT1 and dT2) were used; for the 5' RACE, a perfect match primer (P2) and a 5' anchor primer (AP) were utilized. Sense primers (P3, P4) and antisense primer (P5) were designed for the PCR + 1 and expression cloning reactions. Primer P4 shows the same sequence as primer P3, but has an additional restriction endonuclease site, *Bgl*II at its 5' end; primer P5 was designed to

Abbreviations: Sol i, *Solenopsis invicta*; Sol r, *Solenopsis richteri*; Ves v, *Vespula vulgaris*; PCR, polymerase chain reaction; RT, reverse transcription; RAST, radioallergosorbent test; PBS, phosphate buffered saline, pH 7.4; PIR, protein identification resource; n-, native; r-, recombinant; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; HPLC, high performance liquid chromatography.

Table 1. List of primers used in PCR and PCR + 1

P1	5'-AA(C + T)AA(C + T)GC(A + T + C + G)AT(A + T + C)CA(C + T)AC(A + T + C + G)ATGTG(C + T)CA-3'
P2	5'-TGGAAATGAGGAAATCCTTCGATTATGCA-3'
P3	5'-TATGGAATTAATTGTCAGTATATTA-3'
P4	5'-GAGAGATCTTATGGAATTAATTGTCAGTATATTA-3'
P5	5'-GAGAAGCTTCTA(GTG) ₆ TTTCTTTATTTTCGTATATTGGTGCA-3'
OligodT1	5'-TCTGAATTCTCGAGTCGACATCTTTTTTTTTTTTTTTT-3'
OligodT2	5'-TCTGAATTCTCGAGTCGACATCT-3'
AP	5'-CTGGTTCGGCCACCTCTGAAGGTTCCAGAATCGATAG
An	5'-PO ₃ CACGAATTCATATCGATTCTGGAACCTTCAGAGG-NH ₂

contain six codons for histidine before the stop codon followed by a *Hind*III RE site at the 3' end. The sequences of the primers are listed in Table 1. An additional construct was prepared using the sense primers and an antisense primer similar to P5, but without the six histidine codons.

Isolation of mRNA

Approximately 1 g of whole fire ants, *S. invicta*, were used to isolate mRNA. The isolation was carried out using Poly AT-tract System 1000 (Promega, Madison, WI) following the manufacturer's instructions. The mRNA was alcohol precipitated and stored at -80°C.

Polymerase chain reaction amplification

Reverse transcription polymerase chain reaction (RT-PCR) was used to amplify mRNA and to obtain the cDNA sequence. The cDNA was prepared using oligo dT1 primer and Superscript reverse transcriptase and then purified using glass max columns (Life Technologies, Gaithersburg, MD). The amplification of the 3' fragment was carried out using primer P1 and oligo dT2 primer with 2 mM MgCl₂ and Elongase enzyme mixture (Life Technologies). The PCR was carried out in a MJ research minicycler for 40 cycles: 1 min at 94°C, 2 min at 55°C, and 2.5 min at 72°C. The final polymerization was extended for 10 min. For the 5' fragment, the cDNA was anchored with an oligonucleotide (An) using T4 RNA ligase. The amplification was carried out under the same conditions as for the 3' fragment using the primers AP and P2.

PCR + 1 reactions

The first part of the PCR + 1 reactions (10) were carried out as described before, using P3 and P5 for the amplification. The P3 primer used at 1/10 of the concentration of P5. The PCR fragments were then purified over a Glassmax column. For the second part of this protocol, one half of the first reaction was used as template, and one single cycle was performed using P4 primer and Taq polymerase.

Cloning and screening of PCR and PCR + 1 fragments

The PCR products were analyzed by electrophoresis and cloned into TOPO 2.1 vector (Invitrogen, Carlsbad, CA). After transformation, plasmid DNA minipreparations were prepared and digested with *Eco*RI to confirm positive clones. The identification of complete PCR + 1 products was confirmed by DNA sequencing.

DNA sequencing

Recombinant plasmids were sequenced on an ABI automated sequencer (Applied Biosystems, Foster City, CA) using M13 forward and T7 sequencing primers. Six clones from three independent PCR + 1 reactions were sequenced.

Subcloning of r-Sol i 3 into expression vector pBlueBac III

The coding regions (PCR products of the PCR + 1 reactions) were subcloned from the TOPO 2.1 vector into pBlueBac III (Invitrogen) vector for expression. Briefly, r-Sol i 3 was cut out of the TOPO 2.1 vector using *Bgl*II and *Hind*III RE enzymes and ligated into the *Bam*HI and *Hind*III sites of the pBlueBac III vector. Positive recombinants were confirmed by restriction enzyme digest and gel electrophoresis.

Insect cell cultures

Sf9 cells were grown in Sf900 II serum-free medium from Life Technologies. High-five cells were grown in the serum-free medium Excell 405 (JRH Biosciences, Lenexa, KS). All cells were grown as suspension cultures at 29°C.

Production of recombinant Sol i 3

The recombinant plasmid was cotransfected with linearized wild-type AcMNPV (Invitrogen) into Sf9 insect cells using lysosomes, CellFECTIN (Life Technologies).

Four micrograms of recombinant DNA, 0.5 µg linear AcMNPV and 15 µl liposomes in 1 ml medium were added to log phase Sf9 cells (5×10^6 /60 mm dish) and incubated at room temperature for 5 h. The transfection mixture was then replaced by 2 ml of medium and the cells were incubated for 72 h at 27°C in a humid environment. A plaque purification assay was performed to identify recombinant viruses and confirmed by PCR using baculovirus specific primers (Invitrogen). Further rounds of plaque purification were performed to ensure clonality. High-five cells (1.5×10^6 /ml) were then transfected for production of r-Sol i 3 protein. The supernatant containing the recombinant protein was harvested 4 days post-transfection.

Purification of r-Sol i 3

Purification of r-Sol i 3 was performed using TALON metal resin (Clontech Laboratories, Palo Alto, CA) following the instructions of the manufacturer. Briefly, the cell supernatant containing the recombinant protein was dialyzed against binding buffer (20 mM Tris, 100 mM NaCl pH 8.0) and concentrated about 10-fold. It was then incubated at room temperature for 25 min with the equilibrated Talon resin. The resin was washed three times with 10 bed volumes of binding buffer and two times with 10 bed volumes of binding buffer containing 10 mM imidazole. The protein was eluted with binding buffer containing 100 mM imidazole. The eluted samples were dialyzed against PBS and the protein concentration determined by the Coomassie Plus protein assay (Pierce Chemical Co., Rockford, IL). Further purification was performed by cation exchange chromatography on a Pharmacia (Piscataway, NJ) Mono S column using a gradient of 0–1 M NaCl in 0.02 M acetate buffer, pH 5.2. The purity of the recombinant protein was analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and N-terminal amino acid sequencing. The recombinant protein without the C-terminal hexahistidine was partially purified

by dialysis and gel filtration on a 1.6×95 cm column of Sephadex G-50 fine (Pharmacia) in tris buffered saline, pH 7.4.

Amino acid sequence analysis

Amino acid sequencing was performed on a Milligen-Bioscience 6625 Prosequencer as previously described (2). For peptide mapping proteins were reduced with dithiothreitol in 8 M urea and alkylated with iodoacetic acid. Digestion was performed with Promega modified trypsin at 37°C as previously described (2, 5). Peptides were separated on a 2.1×250 mm HAILS (Higgins Analytical Inc., Mountain View, CA) C18 column with an acetonitrile gradient in 0.1% trifluoroacetic acid (5) and monitored at 220 nm.

SDS-PAGE and immunoblotting

The methods for SDS-PAGE and immunoblotting were performed as previously described (4). Gels were stained with Gel-code Blue (Pierce) and ICN (Irvine, CA) Rapid-Ag silver stain. Immunoblots were developed with a biotin-streptavidin alkaline phosphatase system. The biotinylated anti-mouse immunoglobulin was purchased from Sigma Chemical Co. (St Louis, MO), the streptavidin-alkaline phosphatase from Vector Laboratories (Burlingame, CA) and the BCIP-NBT substrate tablets from Sigma Chemical.

Mass spectrometry

Recombinant Sol i 3 was analyzed on a Perseptive Biosystems Voyager DE RP MALDI-TOF mass spectrometer without internal standards.

IgE reactivity

Cyanogen bromide activated paper disks were coupled with 1 µg of allergen per disk and radioallergosorbent test (RAST) was performed as previously described (11). The RAST inhibition followed our previously described procedure (12).

Circular dichroism

Circular dichroism was performed with a 0.15 mg/ml solution at a path length of 0.1 cm in a PiStar CD Spectrometer from Applied Photophysics, Surrey, UK, at the University of North Carolina Macromolecular Interactions Facility. The data was analyzed using CDPro software from Colorado State University, Fort Collins, CO.

Results

Cloning of Sol i 3

In order to obtain the complete cDNA sequence of the fire ant venom allergen Sol i 3, we utilized RT-PCR, PCR + 1 and standard cloning techniques. Amplifications of the 3' fragments were performed using both oligo dT primers and the degenerate primer P1. The 5' fragments were obtained with matched primers P2 and AP. At least two independent PCR were performed for

both the 3' RACE and 5' RACE. Two independent PCR products were cloned, sequenced and analyzed. In order to avoid sequence artifacts associated with polymorphism, we performed PCR + 1 reactions (10, 13, 14). The primers used for these reactions were designed to amplify the coding region of Sol i 3 and included appropriate restriction sites for expression and six histidine codons at the C-terminal end. We performed three independent PCR + 1 reactions and sequenced one clone from each reaction. The sequences of these three independent clones were identical. The encoding cDNA is 705 nucleotides long to the stop codon and contains a leader sequence of 22 amino acids. The full length nucleotide sequence through the polyA tail and the deduced amino acid sequence are shown in Fig. 1. The coding sequence has been deposited in Genbank with accession number AF012919. The mature protein consists of 212 amino acids and contains eight cysteine residues; furthermore, it shows three potential N-glycosylation sites (N-X-S/T), none of which are glycosylated in the natural molecule (2). Three residues were found to be different from the previously reported amino acid sequence (PIR B37330) (2); re-evaluation of the original peptide sequence data showed ambiguities at these three positions suggesting that the original interpretation was erroneous.

Expression of recombinant Sol i 3

The protein was expressed using an Invitrogen baculovirus expression system. The nucleotide sequence of Sol i 3 has a *Bam* HI site at 310 bp. Therefore, the primers for the amplification of the coding gene were designed to contain a *Bgl*II restriction site on the 5' end and a *Hind*III restriction site after the stop codon. After cloning and sequencing, the product was then subcloned into the expression vector pBlueBac III and cotransfected into Sf9 cells. Recombinant clones were isolated by plaque purification assay with Bluo-gal to detect β-galactosidase producing clones. The plaque purification assay was repeated twice to ensure clonality, as well as confirmed by PCR using baculovirus specific primers (Invitrogen). The production of the recombinant protein was performed using High-five insect cells in suspension culture (1.5×10^6 /ml).

We also used a Baculovirus system from Life Technology, Bac to Bac, with pFastBAC1 as vector. This system eliminates the time-consuming plaque assays. However, we could only produce minute amounts of recombinant protein, which in addition could not be successfully purified by chelating columns.

Purification and characterization of r-Sol i 3

After cation exchange chromatography the purified r-Sol i 3 was free from contaminating proteins as visualized on

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5'  ATG  GAA  TTA  ATT  GTC  AGT  ATA  TTA  TGG  CTT  GCC  ATA  ACA  GCG  GAA  AAC  TTG  GCT
    M   E   L   I   V   S   I   L   W   L   A   I   T   A   E   N   L   A

      63      72      81      90      99      108
AAT  ACA  TTA  GCG  ACA  AAT  TAT  TGC  AAC  CTT  CAA  TCA  TGT  AAG  AGA  AAC  AAT  GCA
    N   T   L   A   ↑  T   N   Y   C   N   L   Q   S   C   K   R   N   N   A

      117     126     135     144     153     162
ATT  CAT  ACG  ATG  TGC  CAA  TAT  ACC  TCA  CCA  ACA  CCG  GGG  CCA  ATG  TGC  TTG  GAA
    I   H   T   M   C   Q   Y   T   S   P   T   P   G   P   M   C   L   E

      171     180     189     198     207     216
TAT  AGC  AAT  GTA  GGT  TTT  ACT  GAT  GCT  GAG  AAA  GAT  GCT  ATA  GTA  AAC  AAA  CAC
    Y   S   N   V   G   F   T   D   A   E   K   D   A   I   V   N   K   H

      225     234     243     252     261     270
AAT  GAA  CTG  AGG  CAG  AGA  GTC  GCA  TCG  GGT  AAG  GAA  ATG  AGA  GGA  ACG  AAC  GGC
    N   E   L   R   Q   R   V   A   S   G   K   E   M   R   G   T   N   G

      279     288     297     306     315     324
CCC  CAA  CCT  CCG  GCG  GTT  AAA  ATG  CCG  AAT  TTG  ACC  TGG  GAT  CCT  GAA  TTA  GCG
    P   Q   P   P   A   V   K   M   P   N   L   T   W   D   P   E   L   A

      333     342     351     360     369     378
ACG  ATT  GCT  CAA  AGA  TGG  GCT  AAT  CAG  TGC  ACG  TTT  GAA  CAT  GAT  GCT  TGT  AGA
    T   I   A   Q   R   W   A   N   Q   C   T   F   E   H   D   A   C   R

      387     396     405     414     423     432
AAC  GTA  GAA  AGA  TTC  GCA  GTA  GGT  CAG  AAT  ATA  GCC  GCA  ACA  TCT  AGC  TCG  GGT
    N   V   E   R   F   A   V   G   Q   N   I   A   A   T   S   S   S   G

      441     450     459     468     477     486
AAA  AAT  AAG  TCC  ACT  CCA  AAT  GAA  ATG  ATT  TTA  TTA  TGG  TAC  AAT  GAA  GTA  AAA
    K   N   K   S   T   P   N   E   M   I   L   L   W   Y   N   E   V   K

      495     504     513     522     531     540
GAC  TTC  GAT  AAT  CGT  TGG  ATT  TCC  TCA  TTT  CCA  TCT  GAC  GAC  AAC  ATT  CTG  ATG
    D   F   D   N   R   W   I   S   S   F   P   S   D   D   N   I   L   M

      549     558     567     576     585     594
AAA  GTA  GGA  CAT  TAT  ACT  CAA  ATT  GTT  TGG  GCT  AAA  ACA  ACG  AAA  ATC  GGG  TGC
    K   V   G   H   Y   T   Q   I   V   W   A   K   T   T   K   I   G   C

      603     612     621     630     639     648
GGA  CGA  ATA  ATG  TTC  AAG  GAA  CCA  GAT  AAC  TGG  ACT  AAA  CAT  TAT  TTA  GTT  TGC
    G   R   I   M   F   K   E   P   D   N   W   T   K   H   Y   L   V   C

      657     666     675     684     693     702
AAC  TAT  GGT  CCA  GCT  GGA  AAT  GTG  CTG  GGT  GCA  CCA  ATA  TAC  GAA  ATA  AAG  AAA
    N   Y   G   P   A   G   N   V   L   G   A   P   I   Y   E   I   K   K

TAG  ATTTGTCATCACCATAATTTCATTTAAAGATGTTCTGATAAGTAATACAATATAAAAAATAATT
    *
    AAAATAAGAACATAAGGTGTATAACAAGGAAAGAAATAAAATGATTTTAAACAGGGAAAAAATAAAAAAATAAAT  3'

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Figure 1. The full length cDNA sequence and translation of the coding region for Sol i 3. This sequence is Genbank entry AF012919. The cleavage of the initiation sequence is shown by the vertical arrow, and the polyA tail initiation signal is underlined.

a Coomassie blue stained gel and by reversed phase high performance liquid chromatography (HPLC) on a HAI-Sil C18 column. The r-Sol i 3 protein eluted from the chelating column formed two major and one minor band in SDS-PAGE. N-terminal amino acid analysis of the mixture and each of the two major components gave the sequence TNYCNLQSKRNNAIHTMXQ which is consistent with mature native Sol i 3. The amount of protein purified varied in individual experiments, but typically gave a yield of 2 mg of pure protein per liter. Cation exchange chromatography separated the r-Sol i 3

into two major and one minor fractions. The first band had slightly higher molecular weight than native Sol i 3, as shown in Fig. 2, because of the hexahistidine tail. The other major band was about 1200 Da larger and a minor band was 2500 Da larger. MALDI-TOF mass spectrometry of the mixed r-Sol i 3 showed a first peak centered at 24 978 Da, in agreement with the calculated molecular weight of 24 793, and a second broader peak centered at 26 145 Da. Tryptic peptide maps of both fractions were almost identical. All of the more intensely absorbing peaks were present at similar intensities in both fractions.

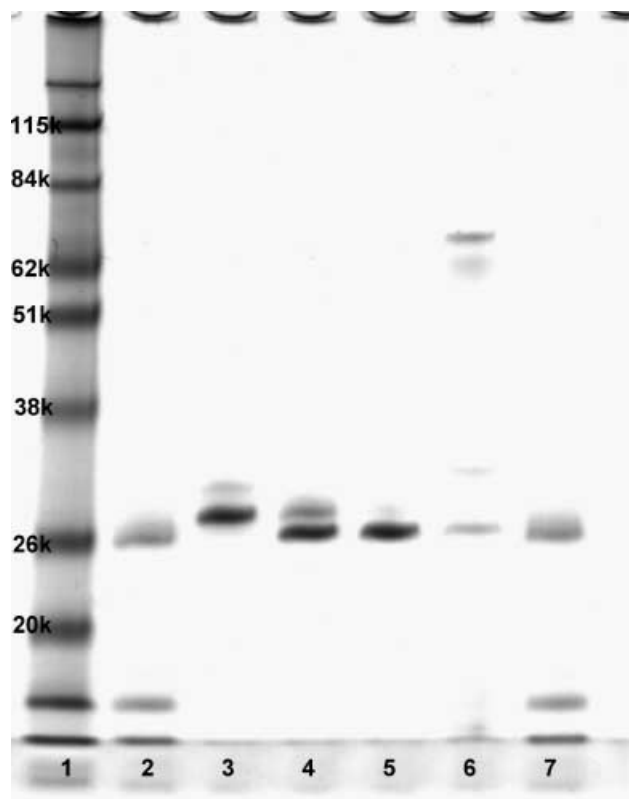


Figure 2. SDS-PAGE of native and fractions of recombinant Sol i 3 after cation exchange chromatography stained with Gelcode blue. Lanes are: 1 – molecular weight standards; 2 – n-Sol i 3; 3 – cation exchange peak 1; 4 – cation exchange peaks 1 and 2; 5 – cation exchange peak 2; 6 – cation exchange exclusion peak; 7 – n-Sol i 3.

A peak was found early in the gradient from the digest of the higher molecular weight form. It was found to have a sequence of modified asparaginyl-lysine. This is consistent with the presence of N-linked carbohydrate at the consensus glycosylation site beginning at residue 124. There are two additional potential N-glycosylation sites at residues 78 and 186, but the tryptic peptides containing these residues each contain tryptophan, which would lead to peaks with very high absorbance at 220 nm on the peptide maps, all of which were identical in maps of both forms of the r-Sol i 3.

Circular dichroism illustrated in Fig. 3 gave very similar curves for both native and recombinant Sol i 3. The alpha helix content of the native molecule was 20.5% compared with a value of 23.5% calculated from the three-dimensional reconstruction; the recombinant showed 16.4% alpha helix with a calculated value of 22.9% without correcting for the additional carbohydrate present in the recombinant molecule. The recombinant molecule showed 6% more beta sheet structure, but similar amounts of turn and disordered structure as the native molecule.

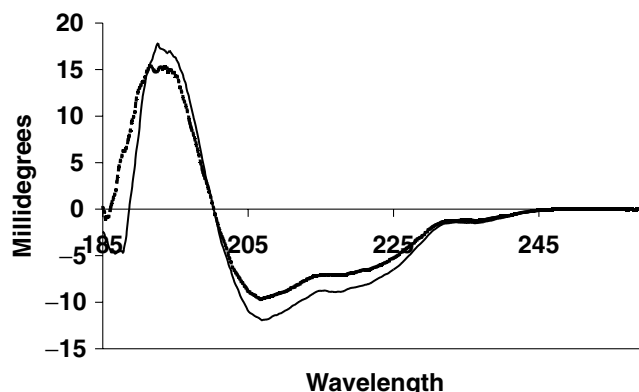


Figure 3. Circular dichroism of native Sol i 3 shown by the thin solid line and recombinant Sol i 3 shown by the heavy dotted line.

Immunologic reactivity of r-Sol i 3

Both forms of the recombinant molecule were strongly reactive in immunoblotting experiments with monoclonal antibody MS2, which binds to native and denatured Sol i 3 (7); however the r-Sol i 3 with C-terminal hexahistidine did not bind to monoclonal antibody AMS32, which reacts only with native conformation Sol i 3. This antibody also does not bind to native Sol r 3 despite its sequence similarity. The sequence differences between Sol i 3 and r 3 are all found in the C-terminal 30% of the molecule (15), suggesting that AMS32 reacts with a C-terminal determinant. A second preparation of r-Sol i 3 without the hexahistidine modification was expressed, and bound to AMS32 in immunoblot experiments with intensity similar to that of native Sol i 3. The modification of recombinant Sol i 3 at the C-terminus by the addition of the hexahistidine tail appears to disrupt the epitope which binds to AMS32.

Radioallergosorbent tests were performed with a panel of 15 individual sera from imported fire ant venom allergic patients. The linear correlation of results between native and recombinant allergens gave $r^2 = 0.98$ as shown in Fig. 4. The RAST inhibition of binding to native Sol i 3 using a pool of 15 sera from patients highly reactive to Sol i 3 indicated that the native and recombinant allergens were highly similar with a relative inhibition constant of 1.3 favoring the recombinant protein (Fig. 5). Maximal inhibition of binding was 89% using 2 μ g of r-Sol i 3.

Discussion

Recombinant allergens have increased our knowledge of IgE-mediated allergies and may be useful in improving the specificity of diagnosis of these diseases (16, 17).

The ways in which glycosylation affect IgE binding are not well studied, but it has been shown that IgE binding is

including its leader sequence. The encoding full-length cDNA was produced using the PCR + 1 method which overcomes artifacts in DNA sequences caused by polymorphism. The protein is 212 amino acid long and is in agreement with the previously reported (2) and corrected (15) protein sequencing results, except for an additional lysine at the C-terminus. For the expression of the protein, we added a hexahistidine tail to the C-terminus of the protein. The C-terminus was chosen to avoid the purification of truncated proteins due to premature termination of translation, as has been observed with other proteins containing an N-terminal hexahistidine tag (24). The protein was expressed in High-five insect cells using a baculovirus system and purified. For expression the natural leader sequence was included, and cleavage at the correct residue was confirmed by N-terminal sequencing of the r-Sol i 3. The purified r-Sol i 3 could bind specific serum IgE from individuals sensitized to fire ant venom as well as a specific monoclonal antibody. The RAST inhibition assay showed 89% inhibition, similar inhibition potencies and slopes, indicating the conformational similarity of r-Sol i 3 and n-Sol i 3, which was further confirmed by circular dichroism spectroscopy. The correlation of positive sera in RAST between the native and recombinant molecules is nearly identical and reinforces the fact that the recombinant protein behaves immunologically like the native protein. The two major forms of the recombinant protein appear to differ by the presence of carbohydrate at asparagine 124. The broad peak in MALDI-TOF mass spectrometry is due to the heterogeneity of the carbohydrate (25, 26). N-glycans from *Trichophusia ni* cells have been reported to contain various mannosylated alpha 1,3- and alpha-1,6-difuco-sylated di-N-acetylglucosamine structures (25, 26). The

N-linked carbohydrate does not appear to have any effect on anti-Sol i 3 binding to the r-Sol i 3 in either immunoblot or RAST assays.

The three-dimensional structure of Sol i 3, modeled using the coordinates for the sequence related structure of *Vespula vulgaris* antigen 5 (27), revealed that major differences occur between the surfaces of the two proteins. The sequences are compared in Fig. 6. A major cleft is present in both proteins, but its shape differs. The insertions found in Sol i 3, compared with Ves v 5 sequence, lead to an extension of surface loops. The different surface structure of these two proteins is consistent with the observed lack of immunologic cross-reactivity (6).

Fire ant venom contains four significant allergenic molecules (1); three of them, Sol i 2, 3 and 4, have now been cloned and expressed (3, 4, 28). These three allergens are all fire ant venom specific with Sol i 2 being the most variable among species (15). Sol i 1, the venom phospholipase (1, 6), has not yet been completely cloned and is the only major fire ant allergen exhibiting cross-reactivity with wasp venom proteins. The combination of Sol i 2 and 3 will detect 100% of individuals with primary fire ant venom sensitization. Sol i 1 is thought to be the major allergen for reactions caused by prior vespid wasp venom sensitization (6).

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References

1. HOFFMAN DR, DOVE DE, JACOBSON RS. Allergens in Hymenoptera venom XX: Isolation of four allergens from imported fire ant (*Solenopsis invicta*) venom. *J Allergy Clin Immunol* 1988;**82**: 818–827.
2. HOFFMAN DR. Allergens in Hymenoptera venom XXIV: the amino acid sequences of imported fire ant venom allergens Sol i II, Sol i III and Sol i IV. *J Allergy Clin Immunol* 1993;**91**:71–78.
3. SCHMIDT M, WALKER RB, HOFFMAN DR, McCONNELL TJ. Cloning and sequencing of cDNA encoding the fire ant venom protein Sol i II. *FEBS Lett* 1993;**319**: 138–140.
4. SCHMIDT M, McCONNELL TJ, HOFFMAN DR. Production of a recombinant imported fire ant venom allergen, Sol i 2, in native and immunoreactive form. *J Allergy Clin Immunol* 1996;**98**:82–88.
5. HOFFMAN DR. Allergens in Hymenoptera venom XXV: the amino acid sequences of antigen 5 molecules. The structural basis of antigenic cross-reactivity. *J Allergy Clin Immunol* 1993;**92**:707–716.
6. HOFFMAN DR, DOVE DE, MOFFITT JE, STAFFORD CT. Allergens in Hymenoptera venom XXI: cross reactivity and multiple reactivity between fire ant venom and bee and wasp venoms. *J Allergy Clin Immunol* 1988;**82**: 828–834.
7. HOFFMAN DR, JACOBSON RS, SCHMIDT M, SMITH AM. Allergens in Hymenoptera Venoms XXIII: the venom content of imported fire ant whole body extracts. *Ann Allergy* 1991;**66**:29–31.
8. STAFFORD CT, WISE SL, ROBINSON DA, HOFFMAN DR. Safety and efficacy of fire ant venom in the diagnosis of fire ant allergy. *J Allergy Clin Immunol* 1992;**90**:653–661.
9. JUNG RC, DERBES VJ, BURCH AD. Skin response to solenamine, a hemolytic component of fire ant venom (*Solenopsis invicta*). *Dermatol Trop* 1963;**5**:241–245.

10. BORRIELLO F, KRAUTER KS. Reactive site polymorphism in the murine protease inhibitor gene family is delineated using a modification of the PCR reaction (PCR + 1). *Nucleic Acids Res* 1990;**18**:5481–5487.
11. HOFFMAN DR. The use and interpretation of RAST to stinging insect venoms. *Ann Allergy* 1979;**42**:224–230.
12. HOFFMAN DR. Allergens in Hymenoptera venom VI: cross reactivity of human IgE antibodies to the three vespid venoms and between vespid and paper wasp venoms. *Ann Allergy* 1981;**46**:304–309.
13. SCHMIDT M, OLSSON S, van der PLOEG I, van HAGE-HAMSTEN M. cDNA analysis of the mite allergen Lep d 1 identifies two different isoallergens and variants. *FEBS Lett* 1995;**370**:11–14.
14. L'ABBE D, BELMAAZA K, DECARY F, CHARTRAND P. Elimination of heteroduplex artifacts when sequencing HLA genes amplified by polymerase chain reaction (PCR). *Immunogenetics* 1992;**35**:395–397.
15. HOFFMAN DR. Reactions to less common species of fire ants. *J Allergy Clin Immunol* 1997;**100**:679–683.
16. GREGOIRE C, CHAPMAN MD. Recombinant allergens. *Clin Rev Allergy Immunol* 2001;**21**:215–227.
17. KAZEMI-SHIRAZI L, NIEDERBERGER V, LINHART B, LIDHOLM J, KRAFT D, VALENTA R. Recombinant marker allergens: diagnostic gatekeepers for the treatment of allergy. *Int Arch Allergy Immunol* 2002;**127**:259–268.
18. O'REILLY DR, MILLER LK, LUCKOW VA. *Baculovirus expression vectors: a laboratory manual*. New York: Oxford University Press, 1994.
19. SMITH GE, JU G, ERICSON BL, MOSCHERA L, LAHM H, CHIZZONITE R, SUMMERS MD. Modification and secretion of human interleukin 2 in insect cells by baculovirus expression vector. *Proc Natl Acad Sci USA* 1985;**42**:8404–8408.
20. SMITH GE, SUMMERS MD, FRASER MJ. Production of human beta interferon in insect cells infected with a baculovirus expression vector. *Mol Cell Biol* 1983;**3**:2156–2165.
21. CHAI H, VASUDEVAN SG, PORTER AG, CHUA KL, OH S, YAP M. Glycosylation and high-level secretion of human TNF-beta in recombinant baculovirus-infected cells. *Biotechnol Appl Biochem* 1993;**18**:259–273.
22. ZARGARI A, SCHMIDT M, LUNDBERG M, SCHEYNIUS A, WHITLEY P. Immunologic characterization of natural and recombinant Mal f 1 yeast allergen. *J Allergy Clin Immunol* 1999;**103**:877–884.
23. OLSSON S, VAN HAGE-HAMSTEN M, WHITLEY P, JOHANSSON E, HOFFMAN DR, GAFVELIN G, SCHMIDT M. Expression of two isoforms of Lep d 2, the major allergen of *Lepidoglyphus destructor*, in both prokaryotic and eukaryotic systems. *Clin Exp Allergy* 1998;**28**:984–991.
24. SOLDATOVA LN, CRAMERI R, GMACHL M, KEMENY DM, SCHMIDT M, WEBER M, MUELLER UR. Superior biologic activity of the recombinant bee venom allergen hyaluronidase expressed in baculovirus-infected insect cells as compared with *Escherichia coli*. *J Allergy Clin Immunol* 1998;**101**:691–698.
25. KULAKOSKY PC, HUGHES PR, WOOD HA. N-linked glycosylation of a baculovirus-expressed recombinant glycoprotein in insect larvae and tissue culture cells. *Glycobiology* 1998;**8**:741–745.
26. TAKAHASHI N, TSUKAMOTO Y, SHIOSAKA S, KISHI T, HAKOSHIMA T, ARATA Y, YAMAGUCHI Y, KATO K, SHIMADA I. N-glycan structures of murine hippocampus serine protease, neuropsin, produced in *Trichoplusia ni* cells. *Glycoconj J* 1999;**16**:405–414.
27. HENRIKSEN A, KING TP, MIRZA O, MONSALVE RI, MENO K, IPSEN H, LARSEN JN, GAJHEDE M, SPANGFORT MD. Major venom allergen of yellow jackets, Ves v 5: structural characterization of a pathogenesis-related protein superfamily. *Proteins* 2001;**45**:438–448.
28. SCHMIDT M, HOFFMAN DR. Expression of recombinant fire ant venom allergen Sol i 4. *J Allergy Clin Immunol* 2001;**107**:S112–S113.