

Sol i 1, the phospholipase allergen of imported fire ant venom

Donald R. Hoffman, PhD,^a Rhonda H. Sakell, BS,^a and Margit Schmidt, PhD^{a,b}
Greenville, NC

Background: Sol i 1, the venom phospholipase of imported fire ant venom is an important allergen and exhibits some cross-reactivity with IgE antibodies from patients sensitized to other Hymenoptera venoms.

Objective: To determine the primary structure of Sol i 1 and evaluate the roles of protein and carbohydrate epitopes in its cross-reactivity.

Methods: Sol i 1 was purified from venom, proteolytic peptides prepared and amino acid sequences obtained. The cDNA for Sol i 1 was cloned, sequenced, and compared with sequences of other wasp venom phospholipases. The role of carbohydrate epitopes in the cross-reactivity with other Hymenoptera venoms was studied by RAST inhibition.

Results: The sequence identified Sol i 1 as a lipase of the GX class, lipoprotein lipase superfamily, pancreatic lipase homologous family and RP2 subgroup phospholipases as are the vespid venom phospholipases. The 148 residues identified by amino acid sequencing represent about 48% of the translated cDNA sequence. Sol i 1 was 31-32% identical to yellow jacket phospholipases. The identical regions of sequence were clustered in the domain which forms the serine hydrolase active site. Mannosylated N-glycans could completely inhibit binding of IgE from honeybee venom sensitized patients to Sol i 1. Inhibition by glycan of IgE binding from yellow jacket venom sensitized patients was low or absent for three of eight sera and substantial, but not complete for five sera.

Conclusions: Sol i 1 is related to wasp venom phospholipases. Cross-reactivity with honeybee venom is caused by carbohydrate, whereas cross-reactivity with yellow jacket venom involves reactivity with both carbohydrate determinants of hyaluronidase and high molecular weight proteins and phospholipase protein determinants. (J Allergy Clin Immunol 2005;115:611-6.)

Key words: Fire ant, phospholipase, venom, cross-reactivity, carbohydrate, allergen

The venom of the red imported fire ant, *Solenopsis invicta*, has been shown to contain 4 important allergens.¹

Abbreviation used

RACE: Rapid amplification of cDNA ends

Sol i 2 and Sol i 4 are related to each other but are unlike proteins described in other *Hymenoptera* venoms. The primary sequences of these allergens have been determined by both amino acid sequencing² and cDNA cloning.^{3,4} Sol i 3 is a member of the antigen 5 family.^{2,5,6} Sol i 2, 3, and 4 have all been expressed by insect cells in immunoreactive form.^{4,5,7} The venom phospholipase, Sol i 1, has been shown to be cross-reactive with IgE antibodies from some patients sensitized to yellow jacket venom.^{8,9} In this study, we have cloned and sequenced the cDNA for Sol i 1, compared the sequence with those of phospholipase A₁B molecules from other *Hymenoptera* venoms, and investigated the nature of the cross-reactivity with IgE antibodies from patients with primary sensitization to yellow jacket and honey bee venoms.

METHODS

Materials

Human sera were used with the approval of the East Carolina University Institutional Review Board. Sera from patients allergic to honeybee and yellow jacket venoms were those reported in our previous study.⁸ Sera were stored at -20°C until used. *S. invicta* were collected locally, flash-frozen, and stored at -80°C until used. Commercial grade *S. invicta* venom was generously provided by Vespa Laboratories (Spring Mills, Pa). Sol i 1 was prepared as previously described.¹ *S. invicta* venom was first separated by gel filtration on a column of Sephadex G-75 superfine (Amersham Pharmacia, Piscataway, NJ) and the high molecular weight protein fraction separated by high performance cation exchange chromatography on a Pharmacia Mono S column in 0.05 mol/L sodium acetate buffer, pH 5.2, with a sodium chloride gradient. The Sol i 1 for protein sequence determination was further purified by reverse-phase chromatography by using a Vydac C4 column (Separations Group, Hesperia, Calif).

SDS-PAGE

Both 1-dimensional and 2-dimensional electrophoresis were performed as we have previously described.¹⁰

Amino acid sequence determination

Peptide mapping and amino acid sequencing was performed as we have previously described.^{2,6} Purified Sol i 1 was reduced with dithiothreitol and alkylated with iodoacetic acid. Digests were performed with sequencing grade trypsin and endoproteinase Lys-C

From ^athe Department of Pathology and Laboratory Medicine, Brody School of Medicine at East Carolina University; and the ^bDepartment of Biology, East Carolina University.

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Reprint requests: Donald R. Hoffman, PhD, Department of Pathology and Laboratory Medicine, Brody School of Medicine at East Carolina University, Greenville, NC 27834. E-mail: hoffmand@mail.ecu.edu.

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purchased from Sigma Chemical Co (St Louis, MO). The digests were separated on a Vydac C18 reverse-phase column. Purified peptides were coupled to AA disks (Millipore Corp, Bedford, Mass) and sequenced on a Milligen-Bioscience Prosequencer (Millipore Corp). N-terminal sequences were determined by using diisothiocyanate disks.

Cloning of the phospholipase

The mRNA was prepared and the cDNA cloned by using methods similar to those we have previously reported.^{3,5} The primers used are shown in Table I. The mRNA was isolated by using the Promega (Madison, Wis) polyAtract system. ELONGase (Invitrogen, Carlsbad, Calif) was used for the PCR reactions and Superscript (Invitrogen) for the RT reactions. Cloning was performed with the Invitrogen Topo TA Cloning Kit. The 3' rapid amplification of cDNA ends (RACE) was performed with primer 181 and 182 and the 5'-RACE with the SMART RACE kit (BD Clontech, Palo Alto, Calif). The sequence of the cDNA was verified by PCR + 1 reaction.¹¹ All sequences were verified by sequencing independently derived clones.

DNA sequencing and analysis

DNA sequencing was performed at the Biomolecular Research Facility of the University of Tennessee at Knoxville. Data processing and analysis were performed by using GCG software (Accelrys, San Diego, Calif), the San Diego Supercomputer Center Biology Workbench (workbench.sdsc.edu), and National Center for Biotechnology Information online resources. Leader sequences were detected with Signal P.¹²

Radioallergosorbent test

The radioallergosorbent test was performed by using our standard procedure.¹³ Specific binding is reported, which is calculated by subtracting the nonspecific binding from total binding. Statistical studies performed in our laboratory for insect venoms show 95% significance of specific binding above 0.5%. RAST inhibition studies were performed as we have previously described.¹⁴ Cyanogen bromide activated paper disks were coupled with 2 µg per disk of purified Sol i 1. The inhibition assay was performed by mixing 50 µL serum with 50 µL borate buffer, pH 8.0, containing the MUXF-BSA inhibitor, and then adding a single Sol i 1 coupled disk.

Three-dimensional structure modeling

Three-dimensional models were constructed by using Swiss-Model software (available at <http://swissmodel.expasy.org/>) with guinea pig lipoprotein lipase 1gpl as template.^{15,16}

Carbohydrate testing

RAST inhibition was performed with synthetic neoglycoprotein fucosylated/xylosylated N-glycans from bromelain coupled to BSA (MUXF-BSA)^{17,18} generously provided by Drs Wolfgang Hemmer and Friedrich Altmann. Venom was absorbed with concanavalin A agarose purchased from Vector Laboratories (Burlingame, Calif), and proteins were eluted with 0.2 mol/L α-methyl-D-mannoside.¹⁹

RESULTS

Amino acid sequencing

Peptides corresponding to 148 amino acid residues were sequenced and used to design the degenerate primers shown in Table I to clone the middle piece of the cDNA. These peptides are shown as the shaded regions in Fig 1, and their sequence identified Sol i 1 as a phospholipase of the lipoprotein lipase group including

TABLE I. Primers

Common	
Oligo (dT) 181	TCTGAATTCTCGAGTCGACATCTTTTTT- TTTTTT
182	TCTGAATTCTCGAGTCGACATCT
Degenerate for middle piece	
SOLI 1 3'B	CRTGRTAYTCNCCYTCRAA
SOLI 1 5'B	TTYATHACNCA YGGNTTYAC
RACE	
3' RACE	CTCGATCCTGCTGATCCTTCCTT
5' RACE	CATCACTCTTGACAGATTCTCTC
PCR + 1	
5'	ATGAGAAAGTTTGCCGCGATATTGT
3'	TTATTGCTGCCTGCACTTAAATAGAT
5' + 1	AGGATCCATGAGAAAGTTTGCCGCGATATTGT

all of the enzyme active site residues. The amino acid sequences of both the N-terminal and C-terminal peptides were determined.

Cloning and sequencing of the cDNA

The middle piece of the cDNA was cloned by using degenerate primers: the forward primer from nucleotides 235 to 254 and the reverse primer from 975 to 993. The sequence was used to choose exact primers for the 3' and 5' RACE, as shown in Table I. The complete clone was prepared and sequenced with the sequence verified by PCR + 1 reaction.¹¹ The results are shown in Fig 1 and are GenBank entry AY684998. The coding region is 1041 nucleotides, corresponding to a protein of 346 amino acids. Analysis using the Signal P program¹² found a signal sequence of 26 amino acids, which was followed by an 11-residue propeptide, similar to that found in the yellow jacket venom phospholipase, Ves v 1.²⁰ The active enzyme contains 309 amino acids with a molecular weight of 34.19 kd, excluding carbohydrate, and a predicted isoelectric point of 8.51. Three potential N-linked carbohydrate sites were found as shown in Fig 1. The enzyme is a lipase of the GX class, lipoprotein lipase superfamily, pancreatic lipase homologous family, and RP2 subgroup phospholipase.²¹

Comparison with wasp venom phospholipases

The alignment of the amino acid sequence of the mature enzyme Sol i 1 with the sequences of 8 other known venom phospholipases is shown in Fig 2. The sequences are GenBank protein AAB48072 for Ves v 1, A44564 for Ves m 1, AAD52615 for Pol a 1, CAA47341 for Dol m 1.01, A44563 for Dol m 1.02, and AAS67041 for Pol d 1.01. The sequences for Ves s 1 and Vesp c 1 are unpublished work from our laboratory. There is 38% identity over 244 residues with Ves m 1, 36% over the same residues with Ves v 1, 40% identity over 206 residues with Dol m 1.01, and 33% over 209 with Dol m 1.02. The longest region of homology is with Pol d 1.01 over 285 residues, whereas there is 35% identity over 209

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1  M R K F A A I F V V F F V Q C T H L Y S 60
1  atgagaaagtttgcgcgataattgtagttttctttgtacaatgcacgcattgtattcc
21  L A Q A R A / E P D P G V V E Y L K / Q S C 120
61  cttgcacaggcagctgcccagcctgatccgggggtgtagagtattgaaacagtcctgt
41  V Y G N S S Y I N V Y L Y N S R F Q G K 180
121  gtctacggtaattctagctacattaacgtatactgtacaacagtcgtttccagggcaaa
61  N L G N Q Q S C Q D I N A S L P V V F I 240
181  aatctcgggaaccaacagagctgccaggatattaatgcacactaccggtcgtcttcata
81  T H G F T S S A Q V S T F K D L A N A F 300
241  actcatggcttcaccagttctgcacaagtatctacatttaagatctggcaaatgcattt
101  V Q K G H T A F I V D W S E A A C T D G 360
301  gtacagaaaggtcatcacagcatttatagtagattggtctgaggcagcttgactgatgga
121  L P G V Q F A E Y N A A A S N T Y D I G 420
361  ctgcccggtgttcaatttgcgcgagtataatgcggcgcatcgaacacttatgatatcgg
141  Q L M A K Y T V D L M N K C K I P L N N 480
421  caacttatggcaaaatataccgttgatttgatgaacaagtgtaaaattccattgaataat
161  I Q Y V G H S L G S H V C G F A A K H V 540
481  atccaatatgtaggtcacagctctggctcacacgtgtgcggcttcgctgcaaaacatgtt
181  K K L I N K T M P Y I L A L D P A D P S 600
541  aaaaaattgataaacaactatgccgtatattcttagcactcgatcctgctgatccttcc
201  F G S N K C G E R I C K S D A K R I V V 660
601  ttccgtagcaataagtgcggggagagaatctgcaagagtgatgccaaacgtatagtagtt
221  F K T S I L G I G E N I I G H L L I V F 720
661  tttaagacgtcgattctcggaataggggagaatattataggccacctcctcatagtgttc
241  D G G K S Q P A C S W Y D V P C S H S E 780
721  gatggtgggaaatcgcaaccggcttctcatggtatgacgttccttgctcacacagcgaa
261  S I V Y A T G M V S G R C Q H L A V P W 840
781  agtatcgatatgcaaccgggatggtgagtggtatgtcaacaccttgcgttctccttgg
281  T A Q Q R I N P I Q W K F W R V F T S N 900
841  acagctcagcagagaatcaaccaattcaatggaaattctggagagttttcacatcaaat
301  I P A Y P T S D T T N C V V L N T N V F 960
901  ataccgcttatctacctctgacacaagaattgtgtagttttgaacaccaatgtattt
321  K N D N T F E G E Y H A F P D C A R N L 1020
961  aagaatgacaatactttcgaaggagaataaccacgcttttctgactgtgcacgaaatcta
341  F K C R Q Q * 1041
1021  tttaagtgcaggcagcaataa
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FIG 1. cDNA sequence and translation for Sol i 1. The yellow and green shaded areas have been verified by amino acid sequencing of peptides. The green shaded regions determine the enzyme active site with the catalytic residues shown enlarged. Underlines indicate potential N-linked glycosylation. The end of the signal sequence is indicated by / and the propeptide cleavage by //. The termination codon is indicated by *.

residues with Pol a 1. As can be seen in Fig 2, approximately 16% to 20% of the residues show conservative changes. Comparison of the precursor molecule sequences of Sol i 1 and Ves v 1 showed 33.7% identity over 332 of the 346 residues. The rooted phylogenetic tree of the venom phospholipase A₁ molecule amino acid sequences produced by using the DRAWGRAM program on the San Diego Supercomputer Center Biology Workbench is shown in Fig 3. As would be expected, Sol i 1 is the farthest evolutionary distance because the others are members of the *Vespidae* family. The fire ant enzyme appears most related to those from *Polistes*.

Construction of a 3-dimensional model of Sol i 1 and comparison with a model of Ves v 1 showed that the core region around the active site is highly conserved in structure, whereas there are substantial variations in other regions of the molecules. As can be seen in Fig 2, there

is an insertion of 12 residues approximately 50 residues from the C-terminal and a deletion of 5 residues near the N-terminal.

Demonstration of carbohydrate on Sol i 1

Commercial grade fire ant venom was diafiltered in 0.1 mol/L ammonium bicarbonate buffer, pH 5.0, and passed through a column of concanavalin A. After extensive washing, the column was eluted with 0.2 mol/L α -methyl-D-mannoside. The eluate was extensively diafiltered and freeze-dried. SDS-PAGE of the eluate, column supernatant, and unfractionated fire ant venom is shown in Fig 4. The eluate is highly enriched in Sol i 1 at approximately 35 kd, whereas the supernatant is depleted compared with the initial venom preparation. This is consistent with the presence of mannosylated glycan on the Sol i 1.

FIG 2. Sequence alignment of Sol i 1 with vespid venom phospholipase allergens. Strictly conserved residues are indicated by *, highly conserved by :, and partially conserved by a . under the alignments. A - indicates an insertion to maximize alignment.

The sequence alignment with vespid venom phospholipases^{20,25} in Fig 2 shows conservation of the regions that form the enzyme active site area but variation in the loops that form some of the outer regions. The 3 cysteines in these outer regions are not conserved between vespid wasp enzymes and fire ant enzyme. Three-dimensional

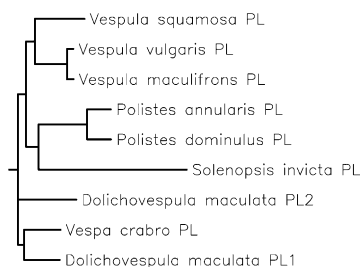


FIG 3. Rooted phylogenetic tree for the amino acid sequences of the 9 phospholipases shown in Fig 2. Horizontal line distances are proportional to calculated phylogenetic differences. PL, Phospholipase.

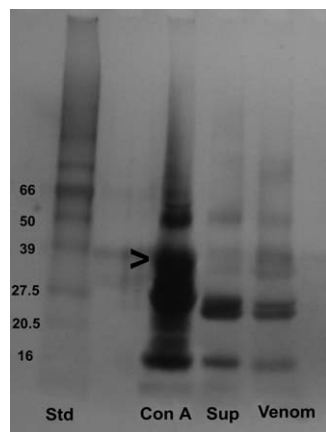


FIG 4. Silver-stained SDS-PAGE gel showing the results of concanavalin A agarose absorption of *Solenopsis invicta* venom. Std is molecular weight standards with values indicated in kilodaltons, Con A is the protein recovered after α -methyl-D-mannoside elution from the column, Sup is the column flow-through that was not absorbed, and Venom is unfractionated *S invicta* venom proteins. The > marks the position of Sol i 1.

computer modeling studies using a guinea pig phospholipase¹⁶ as a template only reconstruct the enzyme core; there is insufficient conservation of the outer loops between the template and Sol i 1.

The phospholipase sequences generally follow the *Hymenoptera* phylogeny. As is shown in Fig 3, the major exception is the 2 isozymes of *Dolichovespula maculata*,²⁶ which are further separated than Dol m 1.01 and Vesp c 1. There are also 2 major sequence variants of *D maculata* antigen 5.²⁷ Sol i 1 is the furthest distant from any of the vespids and appears to be most closely related to *Polistes*. Sol i 3 is similarly placed nearest to *Polistes* in the tree constructed of sequences of antigen 5 molecules (data not shown).

The cross-reactivity with honeybee venom⁸ was expected to result from cross-reactive carbohydrate determinants because honeybee venom does not contain significant amounts of phospholipase A₁, antigen 5, Sol i 2–like, or Sol i 4–like proteins.^{28,29} Immunoblot experiments with sera from yellow jacket–sensitized patients showed cross-reactivity with Sol i 1 but not between Sol i 3

TABLE II. RAST Inhibition with cross-reactive carbohydrate (MUXF-BSA)

	Serum	% Binding to Sol i 1	% Inhibition by MUXF-BSA
Honeybee-sensitized	3867	4.6	100
	3276	4.0	93
	3569	2.6	100
	3590	1.0	100
	3603	1.2	100
Yellow jacket–sensitized	3786	11.4	16
	3543	12.7	87
	3687	14.6	85
	3849	1.5	0
	3723	2.9	79
	3432	2.3	30
Hornet-sensitized	4223	4.3	84
	3850	7.0	100

and antigen 5.⁸ In honeybee venom, the carbohydrate is found on phospholipase, hyaluronidase, protease, acid phosphatase, and some of the higher molecular weight proteins.^{18,28,29} In yellow jacket venom, carbohydrate is found on hyaluronidase and the high molecular weight fraction, but not on the phospholipase.^{10,18,20,28,29} The inhibition studies presented in this article show that although some of the cross-reactivity is a result of cross-reactive carbohydrate determinants, in some sera, a proportion, and in other sera, essentially all of the reactivity is a result of protein determinants. This is consistent with reports that individuals who are known to be sensitized by yellow jacket venom and who have not been exposed to imported fire ant stings can have systemic allergic reactions to their initial fire ant sting.^{8,9}

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