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Crystal Structure of Sol i 2: A Major Allergen from Fire Ant Venom

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Sol i 2 is a potent allergen from the venom of red imported fire ant, which contains allergens Sol i 1, Sol i 2, Sol i 3, and Sol i 4 that are known to be powerful triggers of anaphylaxis. Sol i 2 causes IgE antibody production in about one-third of individuals stung by fire ants. *Baculovirus* recombinant dimeric Sol i 2 was crystallized as a native and selenomethionyl-derivatized protein, and its structure has been determined by single-wavelength anomalous dispersion at 2.6 Å resolution. The overall fold of each subunit consists of five helices that enclose a central hydrophobic cavity. The structure is stabilized by three intramolecular disulfide bridges and one intermolecular disulfide bridge. The nearest structural homologue is the sequence-unrelated odorant binding protein and pheromone binding protein LUSH of the fruit fly *Drosophila*, which may suggest a similar biological function. To test this hypothesis, we measured the reversible binding of various pheromones, plant odorants, and other ligands to Sol i 2 by the changes in *N*-phenyl-1-naphthylamine fluorescence emission upon binding of ligands that compete with *N*-phenyl-1-naphthylamine. The highest binding affinity was observed for hydrophobic ligands such as aphid alarm pheromone (*E*)-β-farnesene, analogs of ant alarm pheromones, and plant volatiles decane, undecane, and β-caryophyllene. Conceivably, Sol i 2 may play a role in capturing and/or transporting small hydrophobic ligands such as pheromones, odors, fatty acids, or short-living hydrophobic primers. Molecular surface analysis, in combination with sequence alignment, can explain the serological cross-reactivity observed between some ant species.

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Abbreviations used: SeMet, selenomethionine; PDB, Protein Data Bank; OBP, odorant binding protein; PBP, pheromone binding protein; cVA, 11-*cis*-vaccenyl acetate; NPN, *N*-phenyl-1-naphthylamine; CSP, chemosensory protein.

Introduction

Fire ants of the genus *Solenopsis* have spread over large parts of the temperate and tropical world from their original habitats in South and Central America.[1] Today, fire ants are serious pests in the southern United States, Pacific Islands,[2] Southeast

Asia,[3,4] and Middle East.[5] In the southeastern United States, the stings of imported fire ants are the most common cause of insect venom anaphylaxis.[1,6,7] Fire ant venom consists of a mixture of very basic piperidine alkaloids[8] and a small aqueous phase containing four major proteins.[9] A single fire ant sting is sufficient to cause specific IgE antibody production, although it contains only 1–10 ng of protein.[1] In some endemic areas, 30–50% of the population shows evidence of sensitization to fire ant venom, as revealed by the presence of serum-specific IgE antibodies and by skin testing.[6]

Four allergenic proteins have been isolated from *Solenopsis invicta* venom and characterized.[9] Sol i 1 is a phospholipase A^1B that is similar to those found in wasp venoms and belongs to the lipoprotein lipase family.[10] It contains carbohydrates and exhibits some cross-reactivity with wasp venom proteins. Sol i 3 is a member of the antigen 5/pathogenesis-related protein family.[11,12] It is not cross-reactive with wasp venom antigen 5, since there is almost no conservation of surface structure.[13] Sol i 2 and Sol i 4 are members of a unique protein family.[9,14,15] Sol i 2 is a covalent dimer composed of two identical monomers. Each monomer contains seven cysteines: six cysteines form three intramolecular disulfide bridges that stabilize the structure, whereas the seventh cysteine (Cys22) links two monomers by a disulfide bridge. Sol i 4 shares a 37% sequence identity with Sol i 2, lacks the dimerizing cysteine, and is present in venom as a monomer rather than as a disulfide-linked dimer. Proteins similar to Sol i 2 are found in venoms of other *Solenopsis* species, including *Solenopsis richteri* (Sol r 2), *Solenopsis geminata* (Sol g 2), and *Solenopsis saevissima* (Sol s 2).[16] Sol i 2 has a high level of sequence identity with Sol r 2 (78.1%), as well as with Sol s 2 and Sol g 2 (71.4%). Although all of the Sol 2 allergens show a high degree of immunological cross-reactivity among the species tested, there are some species-specific determinants in the Sol 2 family of allergens.[16,17] Mouse monoclonal antibodies that are species-specific have been produced, and human IgE cross-reactivity between *S. invicta* and *S. richteri* venoms is not complete.[16,17] Sol i 2 proteins are less stable than Sol i 3 proteins, and their decomposition is responsible for the decay of allergen activity in vaccine extracts.[18]

Only a few three-dimensional structures of insect venom allergens are known to date: the X-ray structure of the major bee venom allergens phospholipase A2[19] and melittin;[20] hyaluronidases from bee venom[21] and wasp venom;[22] antigen 5 from *Vespula vulgaris*[23] and red fire ant;[13] and the complex between bee venom hyaluronidase and its specific IgG Fab.[24] To date, no unique structural feature responsible for the onset of allergenicity has been identified. In this work, the crystal structure of a major allergen from red fire ant (Sol i 2) is reported and compared to its closest structural homologue, that is, the protein LUSH from *Drosophila*, which has specific alcohol and pheromone binding functions *in vivo*.[25,26] The structures of Sol i 2 and LUSH have similar folds consisting of five and six α-helices that surround a central hydrophobic cavity. To verify whether the structural similarity to LUSH also implies functional similarity, we studied the binding of various insect pheromones, repellent, attractants, and plant volatiles to Sol i 2.

Results and Discussion

Structure determination

The crystal structure of the mature recombinant major allergen from fire ant venom, Sol i 2 (residues

Table 1. Data collection and refinement statistics

	P	Ia	Ib
Data collection			
Protein	Native	SeMet	SeMet
Space group	$P2_12_12_1$	$I222$	$I222$
Number of monomers per asymmetric unit	8	2	2
Cell dimensions *a*, *b*, *c* (Å)	110.2, 139.2, 62.1	62.8, 68.7, 111.7	57.5, 67.8, 100.9
Wavelength (Å)	1.0000	0.97930	1.48520
Number of unique reflections	29,059	3638	3739
Resolution (Å)	70–2.58 (2.72–2.58)	58–3.30 (3.48–3.30)	56–3.10 (3.27–3.10)
R_{sym} (%)[a]	5.5 (28.4)	6.4 (68.3)	5.6 (39.3)
Completeness (%)	95.0 (86.3)	90.7 (37.9)	94.4 (62.8)
Redundancy	3.2 (2.3)	16.6 (3.0)	7.0 (2.8)
$I/\sigma(I)$	17.3 (3.8)	24.5 (2.0)	15.6 (1.9)
Refinement statistics			
Resolution range (Å)	15.0–2.6		
Number of unique reflections	26,886		
R_{work}[b]/R_{free}[c] (%)	23.7/27.1		
Number of atoms			
Protein	6872		
Water	79		
Heptane (HP6)	56		
RMSD from ideal values			
Bond lengths (Å)	0.0097		
Bond angles (°)	1.00		
Average *B*-factors (Å^2)			
Protein	37.9		
Water	36.1		
Heptane	33.1		
Ramachandran plot (%)			
Favored region	98.5		
Allowed region	1.5		
Disallowed region	0.0		

The highest-resolution shell is shown in parentheses.

[a] $R_{sym} = \sum |I_i - \langle I_i \rangle| / \sum I_i$, where I_i is the intensity of the *i*th observation and $\langle I_i \rangle$ is the mean intensity of the reflection.

[b] $R_{work} = \sum(||F_o| - |F_c||) / \sum |F_o|)$.

[c] R_{free} = *R* value for a randomly selected subset (5%) of the data that were not used in the refinement.