

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

Donald R. Hoffman, PhD Greenville, N.C.

The most common cause of insect venom allergy in the Southeastern United States is the imported fire ant. The allergens are among the most potent known, with nanogram doses causing sensitization and provoking anaphylaxis. The complete amino acid sequences of imported fire ant venom allergens, Sol i II, III, and IV, were determined by solid-phase protein sequencing of overlapping peptide fragments. Sol i II has a single sequence of 119 amino acids and a molecular weight of 13217. It has seven cysteine residues, and in its native form is a disulfide-linked dimer. The highly purified molecule does not have phospholipase activity and is not structurally related to phospholipases or other known proteins. Sol i IV has 117 amino acids, for a molecular weight of 13340. It has six cysteines and is a monomer. Its sequence is 35% identical to Sol i II, but it is not significantly related to other proteins. The Sol i IV sequence showed two amino acid variations. Sol i III was found to consist of 212 amino acids of molecular weight 24040 in good agreement with 26000 by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The sequence contained eight cysteine residues and was found to be 44% to 50% identical to five vespid venom antigen 5 molecules. IgE antibodies against Sol i III do not exhibit strong cross-reactivity with vespid antigen 5s. The sequence similarity is consistent with other data, suggesting that ants are related to wasps of the superfamily Vespoidea. This sequence data allows future studies to elucidate B- and T-cell determinants and may help to understand the extreme allergenic potency of these proteins. (J ALLERGY CLIN IMMUNOL 1993;91:71-9.)

Key words: Fire ant, *Solenopsis invicta*, amino acid sequence, protein sequence, allergen, Sol i ii, Sol i III, Sol i IV, venom, antigen 5

Since the introduction of the red imported fire ant, *Solenopsis invicta*, into the United States from South America, the ants have spread over the Southeastern states north through the Tidewater area of Virginia and west through more than half of Texas, with isolated colonies in Arizona and New Mexico.¹ The imported fire ant is the most common cause of stinging insect allergy in much of its endemic area,² and sting attack rates have been reported to be as high as 58%

Abbreviations used

<i>Dol a:</i>	<i>Dolichovespula arenaria</i>
<i>Dol m:</i>	<i>Dolichovespula maculata</i>
<i>Pol e:</i>	<i>Polistes exclamans</i>
PTH:	Phenylthiohydantoin
SDS-PAGE:	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
<i>Sol i:</i>	<i>Solenopsis invicta</i>
<i>Ves m:</i>	<i>Vespula maculifrons</i>
CNBr:	Cyanogen bromide
PIR:	Protein Identification Resource
ARG:	Endoproteinase-Arginine-C
V8:	Endoproteinase-Glutamic-C

From the Department of Pathology and Laboratory Medicine, East Carolina University School of Medicine, Greenville.

Supported in part by a grant from The North Carolina Biotechnology Center.

Received for publication April 23, 1992.

Revised Aug. 14, 1992.

Accepted for publication Aug. 24, 1992.

Reprint requests: Donald R. Hoffman, PhD, Department of Pathology and Laboratory Medicine, East Carolina University School of Medicine, Greenville, NC 27858-4354.

1/1/41989

of the population annually.³ The venom is extremely potent allergenically, with IgE antibody responses developing in approximately 25% of exposed individuals.⁴ A typical sting contains from 10 to 100 ng of protein.¹ Systemic reactions can be provoked in highly

TABLE I. Peptides sequenced from *Sol i* II

Cleavage	HPLC fraction	Residues
N-terminal		1-54
Dilute acid hydrolysis		69-105
CNBr	34-1	41-58
	34-2	61-90
Chymotrypsin	19	60-89
	14	47-59
	16-1	10-16
	16-2	106-115
	23	18-36
	7	116-119
	26	6-
	15	41-55
	17	60-89
V8	33	57-73
	49	15-44 +
	23-1	74-86
	27	5-14
	44	87-111
	21	112-119
	25	74-79 +
	47	87-106 +

HPLC, High-performance liquid chromatography.

sensitive individuals by a single sting.⁵ For comparison, a single bee sting contains approximately 50 µg of protein, and vespid wasp stings contain from 1.7 to 17 µg of protein per sting.⁶

Our laboratory has reported on the isolation of three purified allergens, *Sol i* II, III, and IV, and a partially purified fraction *Sol i* I from imported fire ant venom.⁷ These four allergens were shown to not cross-react with each other by studies with human IgE antibodies and mouse monoclonal antibodies; and *Sol i* II, III, and IV did not cross-react with proteins from other stinging insect venoms.⁸ *Sol i* I, however, cross-reacted with yellow jacket venom. Homologous proteins to *Sol i* II and III, and a fraction similar to *Sol i* I were isolated from the venom of the sister species, *Solenopsis richteri*.⁹ In this report we present the complete amino acid sequences of three of the imported fire ant venom allergens, *Sol i* II, III, and IV.

MATERIAL AND METHODS

Venom

Commercial grade imported fire ant venom was a gift from Vespa Laboratories, a division of ALK (Spring Mills, Pa.). Venom allergens were purified by Sephadex G-75 superfine gel filtration and high-performance cation exchange chromatography as we have previously described.⁷ Antigen 5s were prepared from hand milked venom of *Dolichovespula maculata* (*Dol m*), *Vespula maculifrons* (*Ves m*), and *Polistes exclamans* (*Pol e*) as we have previously de-

TABLE II. Peptides sequenced from *Sol i* IV

Cleavage	HPLC fraction	Residues
N-terminal		1-50
Dilute acid hydrolysis		27-64
CNBr		9-40 +
V8	30	111-117
	63a	95-110
	63b	63-94
	80	25-39 +
	29	15-24
ARG	46-1	1-11
	46-2	12-32
	54	106-11
	74	45-50
	27	39-43
	36	19-23 +
	Others	
Chymotrypsin	36	48-57
	46	32-38
	48	19-31
	44	42-47
	38a	39-41
	38b	87-98
	34a	80-86
	28	112-117
	Others	

scribed.¹⁰ Antigen 5 from *Dolichovespula arenaria* (*Dol a*) venom was a gift from Dr. T. P. King. Purity of allergens was assessed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), ELISA with monoclonal antibodies, and N-terminal sequencing runs of 50 to 60 cycles.

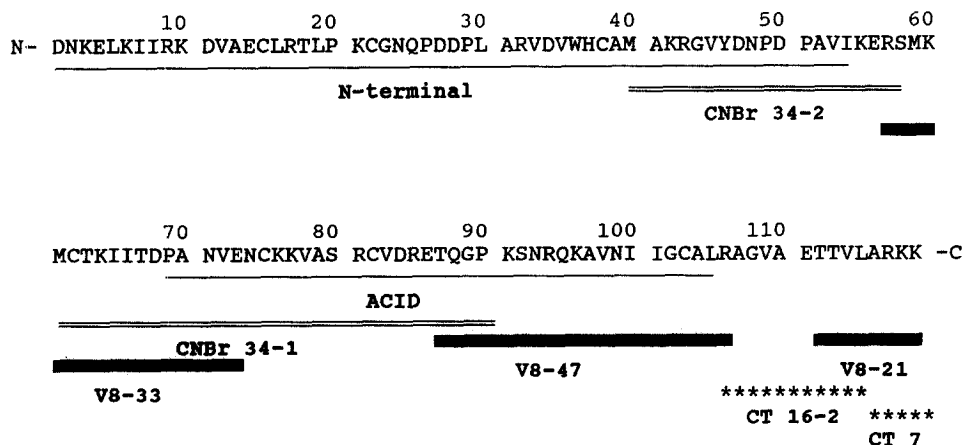
Reduction and alkylation

Purified proteins were reduced in either 6 mol/L guanidine HCl, 0.5 mol/L Tris-HCl, pH 7.4, 0.01 mol/L ethylenediamine tetraacetic acid (EDTA); or 8 mol/L urea in 0.1 mol/L ammonium bicarbonate, pH 7.8, with 5 mg of dithiothreitol under nitrogen for 2 hours at 56° C. The reduced proteins were alkylated with either 13.4 mg of iodoacetic acid (Pierce Chemical Co., Rockford, Ill.) freshly dissolved in 0.1 ml of 0.5N NaOH, in the dark under nitrogen at room temperature for 1 hour, or with 2 µl of 4-vinylpyridine (Sigma Chemical Co., St. Louis, Mo.) for 2 hours under nitrogen at room temperature.¹¹ Denaturing agents and excess reactants were removed by either dialysis or by a Rapidchanger desalting cartridge (Promega, Madison, Wis.).

Protein fragmentation

Cyanogen bromide (CNBr) cleavage was performed in 70% formic acid with at least a 100-fold excess of CNBr, in the dark under nitrogen at room temperature for 24 hours.¹¹ Ten volumes of water were added, and the mixture was dried in a centrifugal vacuum evaporator. Staphylococcal endoprotease-glutamic acid-C (V8) protease digestions were

SEQUENCE : 119 AA.



performed with the insolubilized enzyme kit purchased from Pierce Chemical Co. by following the manufacturer's instructions. Digestions with chymotrypsin (Sigma Chemical Co.) and rat submaxillary protease (endoproteinase-Arginine C [ARG]) (Pierce Chemical Co.) were performed in 0.1 mol/L ammonium bicarbonate, pH 7.8, or Tris-HCl, pH 7.4, with up to 2 mol/L urea added if necessary for solubility, with 2 to 4 μ g of enzyme per milligram of protein at 37° C.^{11, 12} Enzyme hydrolyses were stopped by adding trifluoroacetic acid (Pierce Chemical Co.).

Peptide digests were separated by high-performance chromatography with use of a 25 cm Vydac 218TP54 reversed-phase column (The Separations Group, Hesperia, Ca.) with a gradient starting at 0.1% trifluoroacetic acid in water to 70% of 0.07% trifluoroacetic acid in acetonitrile. The flow rate was 0.6 ml/min and 0.5-minute fractions were collected in microcentrifuge tubes. The separation was monitored at 220 nm.

Sequencing was performed on a Milligen-Bioscience Model 6625 Prosequencer (Millipore Corp., Marlborough, Mass.). This is a solid-phase fully automated sequencer/phenylthiohydantoin analyzer. Proteins were coupled to either diisothiocyanate disks or arylamine disks, and the covalently bound protein was reduced and alkylated in the presence of 6 mol/L guanidine HCl as described above. The disks were then washed five times each in water and methanol and air dried. Separated peptides from digests were coupled to arylamine disks for sequencing. In rare cases peptides were coupled to diisothiocyanate disks to verify aspartic or glutamic acid residues. Acid hydrolysis was performed on covalently coupled protein with 0.1% aqueous trifluoroacetic acid at 110° C for 30 minutes under vacuum. After washing the disk, the peptides with *N*-ter-

The Hitachi Software Engineering America, Ltd. (Brisbane, Calif.) Hibio Prosis programs and compact disk edition of the Protein Identification Resource (PIR) (National Biomedical Research Foundation) and SWISS-PROT (European Molecular Biology Laboratory) data bases were used on an MS-DOS computer system. The program can automatically align pairs of sequences, and a manual alignment routine is provided for multiple sequences. The PIR was release 26.0, and SWISS-PROT was release 15.0.

Quantitative amino acid analysis was performed on HCl vapor hydrolyzed samples of protein with use of the Waters Associates (Marlborough, Mass.) Pico-Tag system modified for a model 600 ternary gradient pump.¹⁴

Sequencing *Sol i* II from the *N*-terminal identified the first 54 residues, and 50 residues were identified from the *N*-terminal of *Sol i* IV. Dilute acid hydrolysis combined with on-line primary amino acid blocking allowed identification of 37 residues of *Sol i* II that did not overlap the *N*-terminus and 38 residues of *Sol i* IV beginning at position 27. Two internal CNBr cleavage peptides were isolated from *Sol i* II residues 40 to 58 and another that could be sequenced for 30 cycles. The only internal CNBr peptide from *Sol i* IV began at position 9. Enzyme hydrolyses were performed on both proteins to isolate a series of overlapping peptides to determine the complete sequence. The peptides sequenced for *Sol i* II are listed in Table

SEQUENCE : 117 AA.

FIG. 2. Complete amino acid sequence of *So/ i* IV shown with single letter amino acid codes and overlapping peptides used to establish the sequence. Cleavage nomenclature is the same as Fig. 1, with the addition of rat submaxillary protease (*ARG*) also called endoproteinase-Arginine-C.

Amino acid	<i>Sol i</i> II count	<i>Sol i</i> IV count
Glycine G	5	5
Alanine A	12	3
Valine V	11	9
Leucine L	6	11 or 10
Isoleucine I	7	13 or 14
Serine S	3	5
Threonine T	6	6
Cysteine C	7	6
Methionine M	3	1
Aspartic acid D	9	4
Asparagial N	7	9
Glutamic acid E	6	6
Glutamine Q	3	1
Arginine R	10	6 or 5
Lysine K	14	18
Histidine H	1	0 or 1
Phenylalanine F	0	2
Tyrosine Y	1	5
Tryptophan W	1	0
Proline P	7	7
Total	119	117
	MW = 13217	MW = 13340

1 and for *Sol i IV* in Table II. The complete amino acid sequences and the key overlapping peptides are shown in Figs. 1 and 2. Amino acid compositions of both allergens are shown in Table III.

In our original description of the properties of *Sol* i II, we found a native molecular weight of approximately 28000 and observed half molecules on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels.⁷ We postulated that this could be due to either proteolysis or acid hydrolysis of the acid labile Aspartic acid-Proline bond. Proteolytic enzyme inhibition did not reduce or prevent half molecule

34.7% identity in 118 aa overlap

	10	20	30	40	50
<i>Sol i</i> IV	DIKEISIMNRILEKCI	RTVPKRENDPIN	PLKNVNLVLYCA	FTKRGIF--	TPKGVNTKQYIN
	: : : : :	: : : : :	: : : : :	: : : : :	: : : : :
<i>Sol i</i> II	DNKELKIIRKDV	AECLRTLPKCGN	QPDPLARVDV	WHCAMA	KRGVYDNPDP
	10	20	30	40	50
	60	70	80	90	100
<i>Sol i</i> IV	YCEKTIISPA	DIKLCKKI	ASKCVKKV	YDRPGPV	IERSKNLL
	: : : : :	: : : : :	: : : : :	: : : : :	: : : : :
<i>Sol i</i> II	MCTKIITDPA	NVENCKK	VASRCVD	RETQGP	KS NRQKA
	70	80	90	100	110

FIG. 3. Computer aligned comparison of amino acid sequences of *Sol i* II and *Sol i* IV. Double dots between sequences indicate identical residues, single dots indicate residues that may differ by a single base, dashes are gaps inserted to maximize alignment. Six cysteine residues are aligned in sequences.

formation. Since the sequence is only 119 amino acids long with a molecular weight of 13217 and contains an odd number of cysteine residues, the molecule apparently is a dimer of identical subunits. The half molecules were generated by incomplete reduction of the dimer. Only a single sequence was observed, with no variation at any position.

The sequence of *Sol i* IV found 117 residues giving a molecular weight of 13340 in good agreement with that found by SDS-PAGE.⁷ Two variations were observed in the sequence and estimated from the quantitation of the sequencer's phenylthiohydantoin analyzer to be in the ratio of approximately 2 to 1. The variants had histidine substituted for arginine at position 23 and isoleucine for leucine at position 37. The heterogeneity was verified by amino acid analysis that showed 0.3 residues of histidine per 13300 molecular weight.

Although *Sol i* II and IV are related to each other by sequence, they appear to be unrelated antigenically with both human IgE and mouse monoclonal antibodies.⁷ Both allergens are clearly distinct from each other, since they do not have any region of sequence with more than three consecutive identical residues as can be seen in Fig. 3. None of the isolated peptides were identical for both allergens. *Sol i* II and IV are also totally unrelated to other known venom proteins from insects of other genera. *Sol i* II is an extremely important allergen in imported fire ant venom, with most allergic patients showing significant reactivity to it. By amino acid analysis it makes up approximately two thirds of the venom protein. Reactivity to *Sol i* IV is less common than to *Sol i* II, and *Sol i* IV only makes up approximately 5% to 9% of the venom protein.⁷ However, when one looks at the allergenic potency of these two proteins in relation to sensitizing and provoking doses, they are both among the most potent allergens known.

The complete amino acid sequence of *Sol i* III was determined from automated solid-phase protein se-

TABLE IV. Peptides sequenced from *Sol i* III

Cleavage	HPLC fraction	Residues
N-terminal CNBr		1-61
	39	180-211 +
	32a	29-59 +
	52	76-113
	65	131-153
	40	180-211
	35	18-
	43a	158-179
	V8	25a
	39a	33-42
	39b	1-25 +
	41	85-99
	18	63-78 +
	23	100-107
	29a	43-52
	48	108-130
	67	131-138
ARG	38	139-158 +
	Others	53-66 +
	48	65-89 +
	40a	12-36 +
	34	197-212
	31	92-104
	26	1-11
	75	92-117 +
	45	57-81 +
	42	65-89 +
	32a	34-54
	44	135-145
	63b	179-188
	Others	

quencing of the intact molecule and purified peptide fragments, isolated by C18 reversed-phase high-performance chromatography of digests by CNBr, endoproteinase-Glutamic acid-C, and endoproteinase-Arginine-C. A list of the peptides isolated and sequenced is shown in Table IV. The complete amino

49.8% identity in 209 aa overlap

	10	20	30	40	50
Sol i III	TNYCNLQSC	RNNAIHTMCQY	TSPTPGPMC-	LEYSNV-GFT	DAEKDAIVNKH
		..		..	
Dol m V.3	NNYCKIK-CSR	--GIHTLCKFG	TSMK-PNCGSK	LVKVHGVSNDE	KNEIVNRHNQF
	20	30	40	50	60
	60	70	80	90	100
Sol i III	SGKEMRG	TNGPQPPAVK	MPNLTWDP	ELATIAQRW	ANQCTFEHD
	..	..			
Dol m V.3	KGLETRG	NPGPQPPAK	NMNVLVW	NDELAKIAQ	TWANQCSFG
	70	80	90	100	110
	120	130	140	150	160
Sol i III	SSSGKNK	STPNEMILL	WYNEVKDF	DNRWISF	PSDDNILMK
		..		..	
Dol m V.3	STTGNSY	ATMSKLIEM	WENEVKDF	NPK---KGT	MGDNNFSK
	130	140	150	160	170
	180	190	200		
Sol i III	IMFKEPD	NWTKHYLV	CNYGPNV	LGA	PIYEIKK
	...	..			
Dol m V.3	VKYIE-NN	WHTHYLV	CNYGPNV	MDQPIY	
	190	200	210		

FIG. 5. Computer-generated comparative alignment of amino acid sequences of *Sol i* III and *Dol m* V.3. *Dashes* indicate gaps inserted to maximize homology. *Two dots* between residues indicate identity, and a *single dot* indicates a single base change. All eight cysteine residues have been aligned. Many of the conserved residues are in groups, for example, from histidine 191 to asparagine 202. There are 104 positions of identity (49.8%) in the 209 residue overlap and an additional 67 (total of 81.8%) positions that could result from single base changes.

data, 1992). Alignment of *Sol i* III with *Dol a* V gave 47.4% match over 211 residues, with *Ves m* V 44.3% over 210, with *Dol m* V.2 46.2% over 212, and with *Pol e* V 44.9% over 214. All of the eight cysteine residues were aligned in each case, and many of the same regions were conserved in all six molecules. In the alignment of *Sol i* III with *Dol m* V.3 an additional 32% of the residues could result from single nucleotide base changes (conservative substitutions).

These results clearly indicate that *Sol i* III is a member of the antigen 5 family of venom proteins. Immunologic studies of IgE antibodies from subjects allergic to imported fire ant venom and from other subjects allergic to vespid venoms and not exposed to imported fire ants did not show the presence of significant cross-reactivity between *Sol i* III and vespid venoms.⁸ The cross-reactivity appeared to be in the *Sol i* I fraction, which contains phospholipase activity. More comprehensive immunologic studies with purified *Sol i* III and vespid antigen 5s may find some limited cross-reactivity.

The amino acid analyses reported in Tables III and V differ somewhat from those we previously reported,⁷ since they are derived from the molecular weights and compositions obtained from protein sequencing. The composition obtained by hydrolysis in Table V was determined by the Pico-Tag method with vapor-phase hydrolysis, whereas the results previously

reported were obtained by liquid-phase hydrolysis and ninhydrin detection.⁷

Ants are classified by various authorities either as the superfamily *Formicoidea* or the family *Formicidae*.¹⁸ In the older classification the superfamily has equal rank to that of the social wasps, *Vespoidea*. More recent investigators using cladistic analysis of multiple types of parameters now place the ant family, *Formicidae*, in the superfamily *Vespoidea*, relatively close to the *Vespidae*, containing yellow jackets, hornets, and paper wasps. Our results strongly support this more recent concept that the ants are closely related to other social wasps.

Knowledge of the amino acid sequences of these allergens should allow the eventual cloning of the genes that code for these proteins and for possible in vitro expression. The sequence allow studies to characterize the T- and B-cell determinants and the potential production of nonallergenic vaccines for specific immunotherapy.¹⁹ As the available data base of sequences of highly allergenic molecules expands, it may become possible to identify those aspects of sequence or structure that are prone to stimulate IgE antibody responses. Although it is traditionally thought that determining protein sequences through molecular biology is much more efficient than by conventional protein sequencing, it appears that sequencing monotypic molecules up to approximately 25000

molecular weight can also be done efficiently and economically by solid-phase automated protein sequencing.

CLINICAL SIGNIFICANCE

The allergens of the imported fire ant are among the most potent allergens known, with nanogram doses typically sensitizing approximately 25% of the exposed population. The three highly purified allergens are all important and do not appear to generate cross-reactive IgE antibodies. Knowledge of the complete amino acid sequence is necessary for attempting to determine the features of these molecules that make them such potent allergens. Knowledge of the sequence is also necessary to elucidate the B- and T-cell reactive determinants of these molecules. Synthetic forms of these determinants can then be used to attempt to modulate the specific immune response to each allergen by reducing specific IgE production and increasing specific IgG blocking antibody production. The observation that *Sol i* III is a member of the antien 5 family of wasp venom allergens, but appears to be non-cross-reactive with vespoid venom antigen 5s, will help us to understand how similar proteins must be to generate cross-reactive IgE antibody responses. In addition, characterization of proteins will eventually allow a much higher degree of control over allergen standardization and quality than is now possible with use of in vivo and immunologic techniques.

REFERENCES

- Stafford CT, Hoffman DR, Rhoades RB. Allergy to imported fire ants. *South Med J* 1989;82:1520-7.
- Stafford CT, Hutto LS, Rhoades RB, Thompson WO, Impson LK. Imported fire ant as a health hazard. *South Med J* 1989;82:1515-9.
- DeShazo RD, Butcher BT, Banks WA. Reactions to the stings of imported fire ants. *N Engl J Med* 1990;323:462-6.
- Stafford CT, Moffitt JE, Bunker-Soler A, Hoffman DR, Thompson WO. Comparison of in vivo and in vitro tests in the diagnosis of imported fire ant sting allergy. *Ann Allergy* 1990;64:368-72.
- 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;90:653-60.
- Hoffman DR, Jacobson RS. Allergens in Hymenoptera venom XII. How much protein is in a sting? *Ann Allergy* 1984;52:276-8.
- 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;82:818-27.
- 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;82:828-34.
- Hoffman DR, Smith AM, Schmidt M, Moffitt JE, Guralnick M. Allergens in Hymenoptera venom XXII. Comparison of venoms from two species of imported fire ants, *Solenopsis invicta* and *richteri*. *J ALLERGY CLIN IMMUNOL* 1990;85:988-96.
- Hoffman DR. Allergens in Hymenoptera venom XIII: isolation and purification of protein components from three species of vespoid venoms. *J ALLERGY CLIN IMMUNOL* 1985;75:599-605.
- Charbonneau H. Strategies for obtaining partial amino acid sequence data from small quantities (<5 nmol) of pure or partially purified protein. In: Matsudaira PT, ed. *A practical guide to protein and peptide purification for microsequencing*. San Diego: Academic Press, 1989:15-30.
- Stone KL, LoPresti MB, Crawford JM, DeAngelis R, Williams KR. Enzymatic digestion of proteins and HPLC peptide isolation. In: Matsudaira PT, ed. *A practical guide to protein and peptide purification for microsequencing*. San Diego: Academic Press, 1989:31-47.
- Andrews DW, Girardi M, Mark J. Chemistry and sequencing of covalently immobilized proteins and peptides. Millipore Corporation, Marlborough, Massachusetts, technical literature MG 345, 1990.
- Bidlingmeyer BA, Cohen SA, Tarvin TL. Rapid analysis of amino acids using pre-column derivatization. *J Chromatogr* 1984;336:93-104.
- Habermann E, Hardt KL. A sensitive and specific plate test for the quantitation of phospholipases. *Anal Biochem* 1972;50:163-73.
- Fang KSY, Vitale M, Fehlner P, King TP. cDNA cloning and primary structure of a white-face hornet venom allergen, antigen 5. *Proc Natl Acad Sci U S A* 1988;85:895-9.
- King TP, Moran D, Wang DF, Kochoomian L, Chait BT. Structural studies of a hornet venom allergen antigen 5, *Dol m* V, and its sequence similarity with other proteins. *Protein Seq Data Anal* 1990;3:263-6.
- Holldobler B, Wilson EO. *The ants*. Cambridge, Mass.: Belknap Press of Harvard University Press, 1990.
- Baldo BA. Structural features of allergens large and small with emphasis on recombinant allergens. *Curr Opin Immunol* 1991;3:841-50.