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Archives of Biochemistry and Biophysics 407 (2002) 224–230

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Structural and immunological aspects of *Polybia scutellaris* Antigen 5

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Received 25 June 2002, and in revised form 13 August 2002

Abstract

Vespid venoms contain Antigen 5, an important allergen whose primary structure and immunological behavior have been extensively studied from venoms of vespids of the Northern Hemisphere. We report herein structural and immunological aspects of Antigen 5 from *Polybia scutellaris* subspecies *rioplatensis* (vulgar name: camoati) found in South America. Mast cell degranulation, histamine release, and IgE induction experiments performed in mice allow us to suggest that *P. scutellaris* Antigen 5 is a variant with reduced IgE response and anaphylactic activity. Sequence data indicate that the protein has a 72.5–90.3% similarity to that of members of the vespid Antigen 5 family with an already known primary structure. Moreover, results suggest that the protein—a new member of an extracellular protein superfamily—could be a good candidate for immunotherapy related to vespid allergy. © 2002 Elsevier Science (USA). All rights reserved.

Keywords: Allergy; Antigen 5; *Polybia scutellaris*; Primary structure; Secretory proteins

Three Hymenoptera families are involved in human allergy: Vespidae (wasps), Apidae (bees), and Formicidae (ants). Among wasps, *Vespula*, *Dolichovespula*, *Vespa*, and *Polistes* are the genera clinically relevant. Most of the patients have reactions to the venom of more than one kind of insect. Hymenoptera venoms are mixtures of three or four proteins—commonly responsible for the allergenic activity—some peptides and small toxins [1].

Vespid venoms contain phospholipases, hyaluronidase, and Ag5,¹ the last being the major and most potent allergen. Ag5—member of a superfamily composed of secreted proteins—is a 23-kDa protein without any known biological activity [2]. Different Ag5s have been isolated and their sequence similarity and allergenic activity widely studied. Data from the first crystallo-

graphic study—corresponding to *Vespula vulgaris* Ag5—have recently been reported [3].

Sequence similarity is 98% for Ag5 from species of the same genus and drops to 57% when Ag5s from lesser related genera—like *Vespula* and *Polistes*—are compared [4]. All of them have some immunological cross-reactivity degree which is relevant to define the composition of mixtures for desensitization treatments [4,5]. Only Ag5 from ant venom does not cross-react with any other Ag5 [6]. All these studies were carried out with Ag5 from insects living in North America and Europe.

Polybia scutellaris is a South American polistinae wasp found mainly in Argentina and Uruguay. The *Polybia* genus is closely related to *Polistes*; however, it is not considered as clinically relevant, on the basis of statistical studies which reported lack of hypersensitivity to its sting in the region where this vespid lives.² However, Cascone et al. [7] isolated Ag5 from *P. scutellaris*

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¹ Abbreviations used: ACN, acetonitrile; Ag5, Antigen 5; CNBr/KI, cyanogen bromide/potassium iodide; PVDF, polyvinylidene difluoride; 4-VP, 4-vinylpyridine; TFA, trifluoroacetic acid.

² Amaral, V. (1996) XVI National Meeting on Allergy and Immunology. “Venenos de Himenópteros de Argentina: estructura y antigenicidad.”

venom and suggested that it could be a lesser- or non-allergenic variant of Ag5. To elucidate the point, we have performed mast cell degranulation assays and measured IgE response to the venom as well as to the purified protein. Besides, we have determined the complete amino acid sequence of this antigen—the first Ag5 reported from a South American vespid venom—as a first attempt to study the structural features supporting its lesser allergenic properties.

Materials and methods

Materials

Polistes venom extract, 4-VP, bovine serum albumin, ovalbumin, dithiothreitol, cyanogen bromide, Coomassie brilliant blue, avidin-peroxidase, orthophenylenediamine, endoproteinase Lys-C, and Staphylococcal V8 proteinase were obtained from Sigma Chemical (USA), and modified trypsin, sequence grade, was from Promega, Madison (WI, USA). Centricon YM-10 filter units and polyvinylidene difluoride membranes were from Millipore (Bedford, MA, USA) and the Immuno-Blot Kit for glycoprotein detection was from Bio-rad (Richmond, CA, USA). Rat monoclonal antibodies were from Caltag Laboratories (Burlingame, CA, USA). BALB/c mice were obtained from the School of Veterinarian Sciences (Buenos Aires University). All other reagents were of analytical grade.

Venom from *P. scutellaris*

Venom was obtained by squeezing sacs from frozen insects and centrifuging the material at 5000g for 15 min. A high-molecular-weight fraction was obtained from the supernatant by using Centricon YM-10 filter units (cutoff: 10,000). Both the venom and the high-molecular-weight fraction were stored at -70°C .

Mast cell degranulation assay

Mast cells were obtained from the peritoneal cavity of Sprague–Dawley rats (male, 250–300 g body weight) [8] after injection of Hank's solution. Cell suspension was diluted to a concentration of 1.5×10^6 cells/ml and aliquots of 50 μl were incubated during 20 min with dialyzed *P. scutellaris* venom as well as with a mixture of dialyzed *Polistes* venom—at the same protein concentration—as the positive control. PBS was used as control for spontaneous degranulation. Mast cell degranulation was evaluated by toluidine blue staining and microscopic examination. On the other hand, the supernatant and cell pellet were separated by centrifugation and the histamine content of both fractions was determined by radioimmunoassay [9].

Immunization of mice

Three groups of 10 BALB/c mice each (8–10 weeks of age) were immunized by biweekly im (crude extract) or ip (purified Ag5s from *P. scutellaris* or *Polistes annularis* containing 3% alum as adjuvant) injections for 4 weeks: the first with 0.3 μg of *Polybia* venom or the purified antigen in 0.1 ml of PBS; the second with 0.3 μg of *Polistes* venom or the purified antigen in 0.1 ml of PBS (positive control), and the third with only 0.1 ml of PBS (negative control). Sera were collected 1 week after the last injection, by retroorbital puncture, and stored at -20°C until used.

ELISA

Microplate wells were coated overnight with rat monoclonal antibodies against mouse IgE in a 0.05 M Tris–HCl, 0.15 M NaCl buffer, pH 7.4, and then treated with 3% powdered milk for 1 h to block nonspecific sites. The wells were then incubated for 1 h with varying dilutions of mouse serum to be tested, 1 $\mu\text{g}/\text{ml}$ of rat monoclonal antibodies to mouse IgE (biotin conjugate) and 1:1000 diluted avidin-peroxidase. Bound peroxidase was detected by absorbance at 490 nm after incubation with a substrate solution (orthophenylenediamine 10 mg/ml, H_2O_2 0.005% in 0.1 M citric acid, 0.1 M $\text{Na}_2\text{PO}_4\text{H}$, pH 5.0).

Purification of Ag5

The method described by Cascone et al. [7] was used. Briefly, 50–100 μl of the ultrafiltered venom was injected in a LKB HPLC system with a 218TP54 (250 $\times$ 3.4 mm) C18 Vydac column. The elution (monitored at 220 nm) was initially isocratic (35% ACN in 0.1% TFA, 10 min), and then a linear gradient (35–55% ACN in 0.1% TFA, 70 min) was used (flow rate, 0.7 ml/min). The purified protein was stored at -70°C .

Amino acid analysis

Samples were hydrolyzed in vacuum-sealed tubes at 110°C for 20 h in constant boiling HCl containing phenol (1 mg/ml) and the hydrolysates were analyzed in duplicate in a Beckman 119 CL analyzer. After performic oxidation, cysteine residues were determined as cysteic acid.

SDS–polyacrylamide gel electrophoresis and electroblotting analysis

SDS–PAGE was performed in slab gels as described by Laemmli [10] and gels were stained with Coomassie brilliant blue. After SDS–PAGE, the Ag5 was electroblotted onto a PVDF membrane according to

Matsudaira [11]. When peptides were submitted to SDS–PAGE, the method of Schagger and von Jagow [12] was used.

Protein concentration

The method of Lowry et al. [13] and bovine serum albumin as the standard were used.

Glycosylation state

The Immuno-Blot kit for glycoprotein detection from Bio-rad was used. Ovalbumin was used as a positive control.

Reduction and alkylation with 4-VP

Protein (50 µg) was reduced in 6 M guanidinium hydrochloride, 0.25 M Tris–HCl, 1 mM EDTA, pH 8.5, with 3.2 mM dithiothreitol, under nitrogen, at 37 °C for 1 h. Alkylation was performed with 1 µl of 4-VP under nitrogen, at 37 °C for 1 h. Reagent excess was removed by RP–HPLC with a 214TP (250 × 4.6 mm) C4 Vydac column.

Cysteine oxidation degree

When the presence of disulfide bridges was investigated, 4-VP was incorporated as described above, before and after protein reduction. Absorbance at 254 nm was then determined.

Reduction and alkylation with iodoacetamide

Ag5 (2.5 µg) was reduced in 6 M guanidinium hydrochloride, 0.25 M Tris–HCl, 1 mM EDTA, pH 8.5, with 10 mM dithiothreitol, under nitrogen, at 42 °C for 1 h. After reduction, iodoacetamide was added to a final concentration of 20 mM and the reaction mixture incubated for 1 h, in the dark, at room temperature. Reagent excess was removed by RP–HPLC with a 214TP (250 × 4.6 mm) C4 Vydac column.

Chemical cleavage

Reduced and 4-VP-treated Ag5 (50 µg) was digested with CNBr/KI [14] in 70% formic acid with a twofold excess of the reactive mixture, in the dark, under nitrogen, at room temperature for 18 h. After dilution with water, the sample was dried in a Speed Vac centrifuge. The peptide mixture was resolved by SDS–PAGE or RP–HPLC by using a Vydac C8 column (30 × 2.1 mm) equilibrated with 5% (v/v) ACN in 0.1% (v/v) TFA. Elution was performed at a flow rate of 0.2 ml/min, with a 5–80% (v/v) ACN, 0.1% (v/v) TFA linear gradient during 20 min.

Endoproteinase Lys-C and staphylococcal V8 proteinase digestions

Approximately 50 µg of the reduced and 4-VP-treated Ag5 was digested with endoproteinase Lys-C or Staphylococcal V8 proteinase, in 0.05 M ammonium bicarbonate, pH 8.2, and a 0.1 M phosphate buffer, pH 7.4, respectively, at an enzyme:Ag5 ratio of 1:20, overnight at 37 °C. Peptide mixtures were resolved by RP–HPLC by using a Vydac C18 column equilibrated with 5% (v/v) ACN in 0.1% (v/v) TFA. Elution was performed at a flow rate of 0.7 ml/min, with a 5–70% (v/v) ACN, 0.1% (v/v) TFA linear gradient over 90 min.

Peptide sequencing

(a) An Applied Biosystems automated pulsed liquid–gas phase sequencer (Model 477, A PE-Biosystems, Foster City, CA, USA) was used. Samples were electroblotted onto a PVDF membrane or applied to a Polybrene-coated filter. (b) A Procise 494 A instrument (PE-Biosystems) was utilized.

Searches for similarities were performed through the Swiss Prot data bank. Peptides were aligned by using the “multiple sequence alignment with hierarchical clustering”.

Tryptic digestion and mass spectrometry

Protein (2.5 µg) reduced and alkylated with iodoacetamide, was submitted to tryptic digestion in 0.1 M ammonium bicarbonate, at an enzyme:Ag5 ratio of 1:20, overnight at 30 °C. The peptide mixture was analyzed by MALDI–TOF–MS using a Bruker Biflex III instrument equipped with delayed extraction and reflector. The sample was prepared by the dried droplet technique using α -cyano-4-hydroxycinnamic acid as the matrix. The spectrum was obtained from 0.5 µl of a 1:1 (v/v) mixture of aqueous matrix solution and peptide mixture. Peptide mass fingerprinting analysis was carried out using ProFound Version 4.7.5. For peptide sequencing, peptides were isolated by microbore RP–HPLC operated in the SMART system (Amersham Pharmacia Biotech, Uppsala, Sweden). A Kromasil C8 column (1 × 150 mm) equilibrated with 0.1% (v/v) TFA was used. Elution was performed at a flow rate of 0.2 ml/min, with a 0–40% (v/v) ACN, 0.1% (v/v) TFA linear gradient for 100 min.

Secondary structure prediction studies

PHD [15] and GOR4 [16] predictive methods as well as the SOPMA consensus prediction from multiple alignments [17] were used. Results were compared with data from the *V. vulgaris* X-ray crystallographic pattern [3].

Results

Biological studies

Results from rat mast cell degranulation showed that, while a major number of cells incubated with *Polistes* venom were degranulated, those incubated with *P. scutellaris* venom, its high-molecular-weight fraction or buffer, maintained their normal morphology; in addition, the number of intact cells in the case of incubation with *P. scutellaris* venom was identical with that of control. Moreover, results from histamine measurements indicated 40.6% of spontaneous release from control mast cells, 60.8% in the presence of *Polistes* venom and 42.8% when cells were incubated with *P. scutellaris* venom. Moreover, results from ELISA assays (Fig. 1A) showed significant differences between their IgE production. While mice immunized with *Polistes*

venom showed a significant IgE increase those immunized with *P. scutellaris* venom reached values similar to those for the negative control group. Additionally, when purified antigens were utilized (Fig. 1B), similar results were obtained, clearly indicating the lower allergenicity of *P. scutellaris* Ag5. These findings led us to study structural aspects of the antigen to find the structural characteristics supporting their different behaviors.

Cysteine oxidation degree

Amino acid analysis after performic acid oxidation indicated the presence of eight cysteine residues. In addition, incorporation of 4-VP before and after protein reduction showed that such cysteines establish four disulfide bridges, which is a common feature in the Ag5 family.

Amino acid sequence

Sequencing of HPLC-purified peptides from the mixtures obtained by Lys-C and V8 protein digestions and their further localization in the sequence by homology with those from other members of the protein family allowed us to determine 62% of the *P. scutellaris* Ag5 primary structure (Fig. 2).

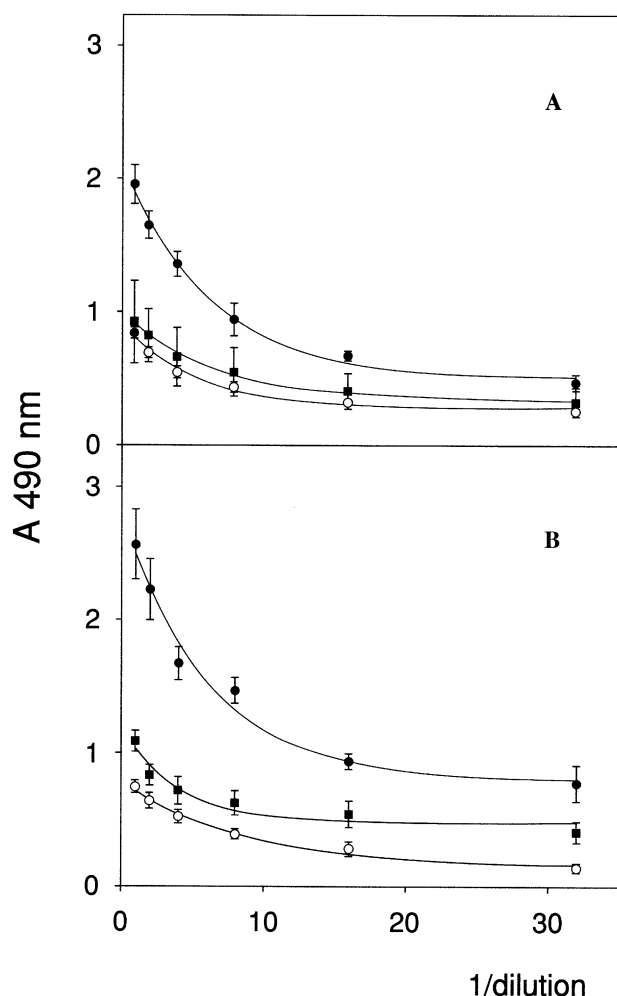


Fig. 1. Results from ELISA assay. *Polistes* (●); *Polybia* (■); control (○). (A) Sera from mice immunized with total venom. (B) Sera from mice immunized with the purified antigens. See Materials and methods.

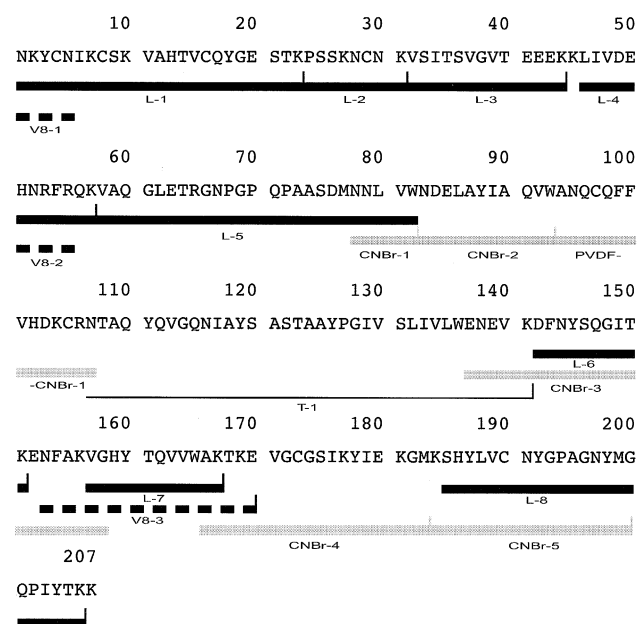


Fig. 2. Amino acid sequence of *P. scutellaris* Ag5. Digestion by Lys-C (L-, wide black full lines) or V8 (V8-, wide black dashed lines) of the reduced and 4-VP-treated protein followed by RP-HPLC; CNBr/KI treatment (wide gray full lines) of the reduced and 4-VP-treated protein followed by RP-HPLC (CNBr-1, 2, 4, and 5); or SDS-PAGE and PVDF electroblotting (PVDF-CNBr-1) and trypsin digestion followed by RP-HPLC (T-, thin black full lines) of the reduced and alkylated with iodoacetamide Ag5. Sequences were determined by Edman degradation and short vertical lines indicate that peptides were fully sequenced.

The main missing region corresponded to a hydrophobic fragment—as judged by sequence comparison between homologous proteins—located between positions 83 and 141. The presence of Met 77, Trp 82, Trp 93, and Trp 136 indicated that the CNBr/KI cleavage could provide useful information. The protein was submitted to this treatment and the peptide mixture resolved by HPLC and SDS–PAGE. An additional 21% of the protein's primary structure was determined; moreover, several crucial overlaps were obtained (Fig. 2). However, segment 107–136 could not be sequenced and, because of the scarce material amount, we had to resort to mass spectrometry to overcome the problem. Reduced Ag5 was alkylated with iodoacetamide and digested with trypsin; peptide mixture was analyzed by MALDI-TOF-MS and separated by RP-HPLC. Peptide mass was compared with the theoretical masses obtained using the sequence of *P. fuscatus* Ag5 for the 107–136 fragment. Only those peptides with a molecular mass different from any of an already-known primary structure were sequenced by Edman degradation. This was the case for a peptide (Fig. 2, T-1) whose sequence indicated its localization at positions 107–141 of the

P. scutellaris Ag5 polypeptidic chain. This result was confirmed by MALDI-TOF-MS since an m/z value of 3799.05 (MH^+ , monoisotopic) was obtained.

Finally, lysine was included at positions 45 and 207 taking into account enzyme specificity and the sequence comparison shown in Fig. 3.

Glycosylation state

P. scutellaris Ag5 is not glycosylated. Despite the data from Swiss Prot and PIR databases indicating that there is no glycosylated Ag5, the presence of a putative glycosylation site in the *P. scutellaris* sequence (Asn 144–Tyr 145–Ser 146) made the analysis important.

Secondary structure prediction studies

Mean values obtained taking into account results from PHD, GOR4, and SOPMA methods indicate that *P. scutellaris* Ag5 would contain 22% α -helix, 22% β -sheet, 7% turn, and 49% coil. Results are highly coincident. Table 1 summarizes these data and shows a comparison with those from the crystalline structure of

	1				39
Polybia	NKYCNKQ.S	KVAHTVCQYG	ESTKPSKNC	NKVSITSVGV	
Polistes	VDYCKIKC.S	SGIHTVCQYG	ESTKPS.KNC	ADKVIKSVGP	
Dolichovespula	NNYCKIKC.S	RGIHTLCKFG	TSMKP...NC	GSKLVKVHGV	
Vespa	NNYCKIKC.R	SGIHTLCKFG	ISTKP...NC	GKNVVKASGL	
Vespula	NNYCKIKCLK	GGVHTACKYG	.SLKP...NC	GNKKVVSYGL	
	40				89
Polybia	TEEEKKLIVD	EHNRFQKVA	QGLETRGNPG	PQPAASDMNN	LVWNDELAHI
Polistes	TEEEKKLIVN	EHNRFQKVA	QGLETRGNPG	PQPAASDMNN	LVWNDELAHI
Dolichovespula	SNDEKNEIVN	RHNQFRQKVA	KGLETRGNPG	PQPPAKNMNV	LVWNDELAHI
Vespa	TKAEKLEILK	QHNEFRQKVA	RGLETRCKPG	PQPPAKSMNT	LVWNDELAHI
Vespula	TKQEKQDILK	EHNDFRQKIA	RGLETRGNPG	PQPPAKNMKN	LVWSDelayI
	90				138
Polybia	AQVWANQCF	FVHDKCRNTA	QYQVGQNIAY	SASTAA.YPG	IVSLIVLWEN
Polistes	AQVWASQCFI	LVHDKCRNTA	KYQVGQNIAY	.AGGSK.LPD	VVSLIKLWEN
Dolichovespula	AQTWANQCSF	.GHDQCRNTE	KYQVGQNVAI	ASTTGNYSYAT	MSKLIEMWEN
Vespa	AQVWAGQCDY	.GHDVCRNTA	KYSVGQNIAY	NGSTAASFAS	VSNMVQMWAD
Vespula	AQVWANQCFY	.GHDTCRDVA	KYQVGQNVAL	TGSTAAVYND	PVKLVKMWED
	139				187
Polybia	EVKDFNYSQG	.ITKENFAKV	GHYTVVWAK	TKEVGCSSIK	YIEKGMKSHY
Polistes	EVKDFNYSNG	.ITKQNFQKV	GHYTMIVAK	TKEIGCGSLK	YMKNNMQHHY
Dolichovespula	EVKDFNPKKG	TMGDNNFSKV	GHYTMVWVGK	TKEIGCGSVK	YIENNNWHY
Vespa	EVKNYQYGST	K...NKLIEV	GHYTMVWAK	TKEIGCGSSIK	YIENGWHRHY
Vespula	EVKDYNPKK	KFSENNFLKI	GHYTMVWAN	TKEVGCSSIK	YIQENWHKHY
	188				207
Polybia	LVCNYGPAGN	YMGQPIYTKK			
Polistes	LICNYGPAGN	YLGQLPYTKK			
Dolichovespula	LVCNYGPAGN	YMDQPIYERK			
Vespa	LVCNYGPAGN	IGNEPIYERK			
Vespula	LVCNYGPSGN	FQNEELYQTK			

Fig. 3. Sequence alignment of *P. scutellaris* Ag5 with consensus sequences of Ag5 from different genera. Sequences and their accession codes were obtained from the SWISS-PROT data bank. *Polistes*, *Dolichovespula*, *Vespa*, and *Vespula* consensus sequences were made with four (*P. annularis*, Q05109; *P. exclamans*, P35759; *P. fuscatus*, P35780; *P. dominulus*, P81656) three (*D. arenaria* Q05108; *D. maculata* 5.01, P10736; *D. maculata* 5.02, P10737), three (*V. crabro* 5.01, P35781; *V. crabro* 5.02: P35782; *V. mandarinia*, P81657), and seven (*V. flavopilosa*, P35783; *V. germanica*, P35784; *V. maculifrons*, P35760; *V. pensylvanica*, P35785; *V. squamosa*, P35786; *V. Vidua*, P35787; *V. vulgaris*, Q05110) already-known sequences, respectively. Consensus sequences were obtained with the amino acids conserved in all the sequences of each genus; when variations were found, the amino acid showing the highest percentage in the sequences was taken. The sequence data for *P. scutellaris* Ag5 have been submitted to the SWISS-PROT bank under Accession No. P82958. Conserved amino acid residues are included in shaded boxes and the *P. scutellaris* sequence numbering was utilized. Dots represent gaps inserted to improve alignment.

Table 1
Comparison between secondary structural elements of *V. vulgaris* Ag5 and those predicted for *P. scutellaris* Ag5

	<i>P. scutellaris</i> Ag5 Secondary structure prediction	<i>V. vulgaris</i> Ag5 crystalline structure
α -Helix ^b		14–17 ^a
	44–60	41–59
	83–94	84–95
	129–141	129–141
		158–163
β -Sheet	3–6	
	13–16	
	32–36	33–37
	99–101	
	117–119	114–122
	160–165	
	175–179	170–180
	186–191	183–193

^a Numbers indicate positions in the *P. scutellaris* Ag5 sequence.

^b α -Helix or β -sheet is indicated only when at least two of the three methods utilized predicted such conformation.

V. vulgaris Ag5; for each residue, either α -helix or β -sheet was selected only when at least two of the three methods indicated such conformational state.

According to Aalberse classification [18] of allergens and the distribution of secondary structure elements of *P. scutellaris* Ag5 similar to those of the *V. vulgaris* Ag5 tertiary structure, the former could be a new member of Family 3 of allergenic proteins.

Discussion

Evaluation of mast cell degranulation activity could be a way to determine the potential allergenic capacity of a substance [19]. On this basis, we have determined the degranulation activity of total *P. scutellaris* venom, as well as its high-molecular-weight fraction, and found that they do not modify mast cell integrity, consistent with the previously described lack of hypersensitivity to the sting of *P. scutellaris*. To further investigate these aspects, we have compared the serum IgE level of mice immunized with *Polistes* or *Polybia* venom. Results indicate that *Polistes* venom is capable of inducing the synthesis of specific-IgE antibodies characteristic of allergens; in contrast, the serum IgE level remains unchanged after inoculation of *Polybia* venom. Moreover, when immunization assays were performed with the purified protein, experiments fully indicate that its behavior hardly differs from that of *Polistes* Ag5, thus denoting its lesser degree of allergenicity.

On the other hand, the sequences of Ag5 isolated from *Polistes*, *Dolichovespula*, *Vespa*, and *Vespula* [4]—all of them being species living in Europe and North America—have been determined. However, the above

results plus the reported lack of hypersensitivity to the sting of *P. scutellaris* make the comparison of its primary structure with known Ag5 structures relevant.

The protein was purified as described by Cascone et al. [7]. Like all other members of the family, the protein is monomeric and has 8 cysteine residues forming four disulfide bridges; it has 207 amino acid residues. Its primary structure shows the seven consensus sequence patterns and the only conserved disulfide bridge described by Henriksen et al. [3] for a superfamily (91 sequences) that includes pathogenesis-related and venom-related extracellular proteins. Total consensus residues (having amino acid residue properties conserved in >90% of the protein superfamily) are also present in *P. scutellaris* Ag5. However, a Val—instead of an amino acid residue involved in turns found in most of the members of the superfamily—is located at position 134.

On the other hand, as shown in Fig. 3, there is a high percentage of similarity (ranging from 72.5 to 85.5%) and identity (from 60 to 77.8%) between the *P. scutellaris* sequence and the consensus sequence of Ag5 from other vespids. The higher values are obtained in comparisons of *Polistes* with *Polybia* Ag5, thus indicating their closely related evolutionary origin. Taking into account all the sequences in Fig. 3, there are 92 conserved residues. Only 4 of those conserved in vespid Ag5 sequences are changed exclusively in that from *P. scutellaris*: at position 5, the sequence has Asn instead of Lys present in the other sequences; at position 110 a Lys is changed for Gln. Val is located at position 11 in *P. scutellaris* Ag5 instead of Gly appearing in the remaining structures; the change of Gly for any other amino acid residue can lead to a local rigidification of the chain, which may bring about major differences in the local conformation of homologous proteins. There is Val instead of Met at position 163; this change does not seem relevant since both amino acids have similar chemical characteristics. Finally, a Ser insertion is present at position 25. Three of these changes are located in the protein N-terminal area (Asn 5, Val 11, and Ser 25). King et al. [20] have reported that the dominant B cell epitopes of *V. vulgaris* Ag5 are precisely in the N-terminal region; this is consistent with the fact that nearly all residues in its N-terminal area are accessible to the solvent. Our work on a molecular model of *P. scutellaris* Ag5 based on the crystal structure of the *V. vulgaris* protein (the only Ag5 whose 3D structure is known) is in progress.

On the other hand, determination of the complete protein sequence will allow us to use synthetic peptides in order to contribute to the determination of allergen epitopes.

It is very likely that the use of variants with reduced or eliminated anaphylactic activity still retaining immunogenic properties will lead to a further improvement in the diagnosis and specific therapy for type I allergens

in the near future [20,21]. *P. scutellaris* Ag5 is a natural variant with reduced allergenic activity and could be considered a good option for specific immunotherapy.

Acknowledgments

We are greatly indebted to Dr. Verónica Amaral for supplying the *P. scutellaris* crude extract. *P. annularis* Ag5 was kindly provided by Dr. Te Piao King (Rockefeller University). We also acknowledge the expertise of D. Beatti, E. Dacci, and S. Linkens. We thank Professor Rex Davis for language supervision of the manuscript. This work was supported in part by grants from the Universidad de Buenos Aires.

References

- [1] D.R. Hoffman, in: M.I. Levine, R.F. Lockey (Eds.), Monograph on insect allergy. American Academy of Allergy & Immunology, Dave Lambert Associates, Pittsburgh, 1995, pp. 27–38.
- [2] T.P. King, D. Moran, D.F. Wang, L. Kochoumian, V.T. Chait, Protein Seq. Data Anal. 3 (1990) 263–266.
- [3] A. Henriksen, T.P. King, O. Mirza, R. Monsalve, K. Meno, H. Ipsen, J.N. Larsen, M. Gajhede, M.D. Spangfort, Proteins 45 (2001) 438–448.
- [4] D.R. Hoffman, J. Allergy Clin. Immunol. 92 (1993) 707–716.
- [5] G. Lu, M. Villalba, M.R. Coscia, D.R. Hoffman, T.P. King, J. Immunol. 150 (1993) 2823–2830.
- [6] D.R. Hoffman, J. Allergy Clin. Immunol. 91 (1993) 71–79.
- [7] O. Cascone, V. Amaral, P. Ferrara, N. Vita, J.C. Guillemot, L. Díaz, Toxicon 33 (1995) 659–665.
- [8] G.A. Mackay, F.L. Pearce, Int. Arch. Allergy Immunol. 109 (1996) 258–265.
- [9] N. Engel, G. Cricco, C. Davio, G. Martín, M. Croci, C. Fitzsimons, R. Bergoc, E. Rivera, Inflamm. Res. 45 (1996) 64–68.
- [10] U.K. Laemmli, Nature 227 (1970) 680–687.
- [11] P.T. Matsudaira, in: P.T. Matsudaira (Ed.), A practical guide to peptide and protein purification for microsequencing, Academic Press, Inc., San Diego, 1989, pp. 52–66.
- [12] H. Schagger, G. von Jagow, Anal. Biochem. 166 (1987) 368–379.
- [13] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, J. Biol. Chem. 193 (1951) 265–275.
- [14] S.S. Huang, J.S. Huang, J. Protein Chem. 13 (1994) 450–451.
- [15] B. Rost, C. Sander, J. Mol. Biol. 232 (1993) 584–599.
- [16] J. Garnier, J.-F. Gibrat, B. Robson, in: R.F. Doolittle (Ed.), Methods in Enzymology, vol. 266, Academic Press Inc., San Diego, 1996, pp. 540–553.
- [17] C. Geourjon, G. Deleage, Comput. Appl. Biosci. 11 (1995) 681–684.
- [18] R.C. Aalberse, J. Allergy Clin. Immunol. 106 (2000) 228–238.
- [19] T. Dudler, D. Cantarelli-Machado, L. Kolber, R. Annand, N. Rhodes, M. Gelb, E. Koelsch, M. Suter, B. Helm, J. Immunol. 155 (1995) 2605–2613.
- [20] T.P. King, S.Y. Jim, R.I. Monsalve, A. Kagey-Sobotka, L.M. Lichtenstein, M.D. Spangfort, J. Immunol. 166 (2001) 6057–6065.
- [21] R. Valenta, S. Vrtala, S. Laffer, D. Kraft, Allergy 53 (1998) 552–561.