Two novel inflammatory peptides were isolated from the venom of the social wasp *Polybia paulista*. They had their molecular masses determined by ESI-MS and their primary sequences were elucidated by Edman degradation chemistry as:

- *Polybia-MPI*: IDWKKLLDAAKQL-NH₂ (1654.09 Da),
- *Polybia-CP*: ILGTILGLKSL-NH₂ (1239.73 Da).

Both peptides were functionally characterized by using Wistar rat cells. *Polybia-MPI* is a mast cell lytic peptide, which causes no hemolysis to rat erythrocytes and presents chemotaxis for polymorphonucleated leukocytes (PMNL) and with potent antimicrobial action both against Gram-positive and Gram-negative bacteria. *Polybia-CP* was characterized as a chemotactic peptide for PMNL cells, presenting antimicrobial action against Gram-positive bacteria, but causing no hemolysis to rat erythrocytes and no mast cell degranulation activity at physiological concentrations.

© 2005 Elsevier Inc. All rights reserved.

Keywords: *Polybia paulista*; Hymenoptera insect; Polycationic peptide; Wasp venom; Mastoparans; Chemotactic peptides

1. Introduction

Stings by Hymenoptera insects such as hornets, yellow jackets and honeybees are manifested by symptoms of pain, local edema and cardiovascular disturbances [5]. The Hymenoptera venoms are constituted of biogenic amines (histamine, serotonin, dopamine, and norepinephrine), some proteins (phospholipases, hyaluronidase, antigen S) and a series of biologically active polycationic peptides such as: mastoparans, mastokinetic peptides and waspkinins among the wasps; mellitin, MCD-peptide and apamine among the bees [1,15]. Mast cell degranulating peptides and chemotactic peptides are among the major components of vespid venoms [13]. The mastoparans are amidated tetradecapeptides responsible for histamine release from the mast cells, of serotetone from platelets, of catecholamines and adenylcy acids from adrenal chromatin cells [24] and even causes exocytosis in rat pituitary cells [26] and pancreatic β-cells [34]. Mastoparans are thought to cause the formation of ion channels in lipid membranes leading to cell lysis [17], and they are also known to increase permeability of ions and small molecules through the biological membranes by...
forming pores at high peptide concentrations [25]. These peptides also promote an increase of intracellular Ca$^{2+}$ concentration in neutrophils [27]. Investigations directed toward elucidation of the regulatory mechanism of mastoparan have shown that these peptides are involved with the modulation of the activities of proteins as diverse as GTP-binding proteins (G proteins), phospholipase A$_2$, and phospholipase C [10,32].

The second most important group of biologically active peptides from wasp venom are the chemotactic peptides, which are tridecapeptides presenting many hydrophobic amino acid residues and generally a single basic residue; they generally attract macrophages and polymorphonuclear leukocytes to the region around the site of stinging [33]. Depending on their primary sequences, these peptides can cause hemolysis and exhibit a reduced activity of mast cell degranulation [11,23,12].

Polycationic peptides usually contain from 12 to 50 amino acids residues, and a net positive charge from +2 to +7 due to the excess of basic amino acid residues; the hydrophobic residues represent more than 50% of their amino acid sequences [8]. These structural features contribute to the formation of amphipathic, $\alpha$-helical conformations, making them able to interact with the anionic components of the bacterial membranes, providing their assembly in these membranes with consequent pore formation [7]. Because of this, some of these peptides also can present antimicrobial activities [15,19]. The amphipathic, $\alpha$-helical conformations also may permit the assembling of some of these cationic peptides with the zwitterionic membranes of mammalian cells, making some of these peptides act as hemolysins of this cells [15].

Recently, novel pharmacologically active peptides from tropical wasp venoms have been reported: three novel mastoparan peptides were described in the venom of Protopolybia exigua, with two of them characterized as modulators of mast cell degranulation by virtue of their interaction with G-protein receptors [21]. Micro-scale bioassay guided low abundant inflammatory peptides, naturally occurring in the venom of the wasp Agelaia pallipes pallipes were sequenced by using tandem mass spectrometry protocols, permitting the identification of novel mastoparan and chemotactic peptides [20]. Two novel inflammatory peptides were described in the venom of the social wasp Polistes paulista, presenting acetylation at the N-terminus, responsible by the modulation of the PMNL cells chemotaxis and histamine release from mast cells [28].

In the present work we report two novel inflammatory peptides isolated from the venom of the social wasp P. paulist$\acute{a}$. They were purified by HPLC under reversed phase conditions, had their molecular masses determined by ESI-MS and their sequences were determined by automated Edman de novo sequence chemistry. Their functional characterization revealed that both peptides exhibit inflammatory and antimicrobial activities.

### 2. Materials and methods

#### 2.1. Sample preparation

The wasps collected in Rio Claro-SP, southeast Brazil, were immediately frozen and stored at $-20$ °C. The venom reservoirs of 3000 worker wasps were removed by dissection with surgical microscissors and washed with 1:1 acetonitrile (MeCN, Aldrich): water containing 0.1% (v/v) trifluoroacetic acid (TFA, Aldrich) to solubilize the peptides. The extract was then centrifuged at 8000 $\times$ g during 15 min at 4 °C; the supernatant was collected and used to purify the peptides.

#### 2.2. Peptide purification

The supernatant described above was chromatographed in a CAPCELL PACK C-18 UG120 column (10 mm $\times$ 250 mm, 5 $\mu$m, Shisheido) under a linear gradient from 5 to 60% (v/v) MeCN [containing 0.1% (v/v) trifluoroacetic acid], at a flow rate of 2.0 mL/min over 60 min by monitoring at UV 214 nm. The extracts were manually collected and dried by using a lyophilizer (MLW LGA-05, Heto). The peaks of interest (fractions 11 and 12) were re-chromatographed under reversed phase chromatography with the same column described above, by using isocratic elution with 41% (v/v) MeCN for the fraction 11 and 45% (v/v) MeCN for the fraction 12 (containing 0.1% TFA in both situations) at a flow rate of 2.0 mL/min during 20 min at 30 °C. The elution was monitored at 215 nm and fractions were manually collected in 5 mL glass vials.

#### 2.3. ESI mass spectrometry analysis

All the mass spectrometric analysis were performed in a triple quadrupole mass spectrometer (MICROMASS, mod. Quattro II). The experimental protocol was based in details described in a previous publication [19] and adapted for the present investigation. The mass spectrometer was outfitted with a standard probe electrospray (ESI—Micromass, Altrincham). The samples were injected into electrospray transport solvent by using a micro syringe (500 $\mu$L) coupled to a micro infusion pump (KD Scientific) at a flow rate of 4 $\mu$L/min.

The mass spectrometer was calibrated with intact horse heart myoglobin and its typical cone-voltage induced fragments to operate at resolution 4000. The samples were dissolved in 50% (v/v) acetonitrile [containing 0.1% (v/v) formic acid] to be analyzed by positive electrospray ionization (ESI$^+$) using typical conditions: a capillary voltage of 3.5 kV, a cone voltage of 30 V, a desolvation gas temperature of 80 °C and flow of nebulizer gas (nitrogen) about 15 L/h and drying gas (nitrogen) 200 L/h. The spectra were obtained in the continuous acquisition mode, scanning from m/z 100 to 2500 at a scan time of 5 s. The acquisition and treatment of data were performed with MassLynx software.
2.4. Amino acid sequencing

The amino acid sequence was performed by using a gas-phase sequencer PPSQ-21 A (Shimadzu) based on automated Edman Degradation Chemistry.

2.5. Biological activities

2.5.1. Mast cell degranulation activity

Degranulation was determined by measuring the release of the granule marker, β-glucosaminidase, which co-localizes with histamine, as previously described [9]. Mast cells were obtained by peritoneal washing of female adult Wistar rats. The mast cells were washed three times by re-suspension and centrifugation in a mast cell medium (150 mM NaCl (MERCK), 4 mM KCl (MERCK), 4 mM NaH2PO4 (SYNTH), 3 mM KH2PO4 (SYNTH), 5 mM glucose (SYNTH), 15 μM BSA (SIGMA), 2 mM CaCl2 (MERCK), 50 μL/L Laqueumine (5000 U/1.250 mL, ROCHE)).

The cells were incubated with various peptide concentrations for 15 min at 37 °C and after centrifugation the supernatants were sampled for β-glucosaminidase. Briefly, 50 μL substrate, 5 mM p-nitrophenyl-α-acetyl-β-glucosaminide in 0.2 M citrate, pH 4.5, and 50 μL of the samples of the medium were incubated in 96-well plates for 6 h at 37 °C to yield the chromophore, p-nitrophenol. After incubation, 50 μL of the previous solution were added to 150 μL of 0.2 M Tris and absorbance was measured at 405 nm. The values were expressed as the percentage of total β-glucosaminidase activity from rat mast cell suspensions, determined in lysed mast cells in presence of 0.1% (v/v) Triton X-100 (considered as 100% reference). Results are expressed as mean ± S.D. of five experiments.

2.5.2. Mast cell lysis

This method measures the leakage of lactate dehydrogenase (LDH, EC 1.1.1.27) from the cytoplasm of rat mast cells into the surrounding medium as an indicator of mast cell lysis by the peptide toxins.

LDH catalyzes the reversible reduction of pyruvate to lactate with NADH as coenzyme. The activity of lactate dehydrogenase was assayed with the supernatants of rat peritoneal mast cells incubated with the toxin peptides also used for mast cell degranulation assay as described above (Section 2.5.1). The LDH activity was assayed by using the UV-LDH Assay Kit from Biobras Diagnostics. 20 μL each supernatant was pre-incubated with 800 μL of LDH buffer (50 mM Tris pH 7.4, containing 1.2 mM pyruvate, 5 mM EDTA) during 5 min, at 25 °C. The standard spectrophotometric LDH assay measured the decreasing of absorbance at 340 nm due to the consumption of NADH in this reaction.

The reaction was initiated by the addition of 200 μL of LDH substrate (0.15 mM NADH), the kinetics of NADH consumption was monitored by acquiring the decreasing of the absorbance at 340 nm during 12 min (ΔA340) at 25 °C. The results were initially calculated as catalytic units (micromoles of NADH min⁻¹ at 25 °C and pH 7.4) and then converted into relative activity by using the total LDH activity of rat mast cells lysed in presence of 0.1% (v/v) Triton X-100 (considered as 100% reference). Results are expressed as mean ± S.D. of five experiments.

2.5.3. Hemolytic activity

Five hundred microliters of washed rat red blood cells (WRRBC) were washed three times with physiological saline solution, and suspended in 0.9% NaCl solution (physiological saline solution) and then counted by using a Neubauer chamber under a microscope after violet crystal staining. Results are expressed as mean ± S.D. of five experiments.

2.5.4. Chemotaxis assays

Chemotaxis was assayed in a specific multi-chamber apparatus (NEURO PROBE) [6] by using polymorphonucleated leukocytes (PMNL), obtained from subcutaneous inflammatory induction in Wistar rats. The upper chambers were filled with 200 μL of a PMNL suspension (~2.7 × 10⁶ cells/mL) in 0.9% NaCl solution (physiological saline solution) and the lower chambers were filled with 400 μL of physiological saline solution containing various concentrations of the peptides (10⁻³ to 10⁻⁴ M). A polycarbonate membrane containing pores of 10 μm of diameter (NEURO PROBE) was placed between both chambers. The chemotaxis chamber was incubated at 37 °C for 1 h. After incubation, cells in the lower chamber were counted by using a Neubauer chamber under a microscope after violet crystal staining. Results are expressed as mean ± S.D. of five experiments.

2.5.5. Antimicrobial activity

The minimal inhibitory concentrations (MIC) of the peptides were determined based on methods previously described elsewhere [2, 18]. The microorganisms used were: Bacillus subtilis (CCT 2576), Staphylococcus aureus (ATCC 6538), Escherichia coli (ATCC 25922) and Pseudomonas aeruginosa (ATCC 15422).

The assays were performed in 96-well plates. The inocula were prepared in saline solution (0.9% (v/v) NaCl) by suspending a 18 h old culture in Müller-Hinton broth to the 0.5 value of a McFarland scale, a concentration equivalent to 1 × 10⁵ colony-forming units (CFU)/mL and 50 μL were inoculated into the wells, containing 50 μL of solution of each peptide solubilized in Müller-Hinton broth medium. The concentration range of the peptides was from 0.5 to 1000 μg/mL.

The plates were incubated at 37 °C for 24 h and after 20 μL of a triphenyltetrazolium chloride solution (TTC) 0.5% (w/v) was added. The plates were then incubated for an additional
period of 2 h at 37 °C. The minimal inhibitory concentration was that where the dye was not reduced and tetracycline in a concentration range from 0.2 to 600 μg/mL was used as control. Results are expressed as mean ± S.D. of five experiments.

3. Results

3.1. Peptides purification and sequencing

The crude venom of *P. paulista* was initially fractionated under reversed-phase-HPLC by using a linear gradient from 5 to 60% (v/v) acetonitrile [containing 0.1% (v/v) trifluoroacetic acid]. The chromatographic profile (Fig. 1) shows the existence of 13 peaks, which were submitted to preliminary biological assays. Fractions 1 and 2 were constituted by complex mixtures of endogenous biogenic amines and neurotransmitters, while fractions 3–12 contained unidentified venom components; the fraction 13 consisted of a mixture of two acetylated inflammatory peptides already characterized both structurally and functionally [28] and unknown venom components. The fraction 11 presented some mast cell degranulation, chemotaxis for PMNL cells and potent inhibitory activity against both Gram-positive and Gram-negative bacteria, whereas the fraction 12 presented some mast cell degranulation, chemotaxis for PMNL cells and potent inhibitory activity against Gram-positive bacteria. Afterward, the purity of fractions were tested by ESI-MS. These observations lead to further purification of the peptide components of these fractions by reverse-phase HPLC under isocratic conditions (Fig. 2 a and b). The biological activities described above were found associated to the peaks 11C (Fig. 2 a) and 12A (Fig. 2 b); ESI-MS spectra revealed the high purity of the isolated peptides with molecular ion peaks at m/z 1654.09 and 1239.73 Da for fractions 11C and 12A, respectively (not shown results). Therefore, both peptides were considered pure enough to be sequenced by Edman Degradation Chemistry and to be biologically characterized.

The primary sequences determined for both peptides were:

- **Fraction 11C**: IDWKKLLDAAKQIL-NH₂ (1654.09 Da)
- **Fraction 12A**: ILGTLGLLKS-NH₂ (1239.73 Da)

The sequences above just fit to the experimental values of the respective molecular masses if the C-terminal residues were considered in the amidated form, as with most of the peptide toxins from the venoms of Hymenoptera [14,30]. The primary sequence of the peptide present in the fraction 11C was compared to other similar peptides from the venom of social wasps as shown in Table 1, revealing some

Table 1: Amino acid sequences of *Polybia-MPI* compared to other mastoparan peptides from social wasp venoms

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Primary sequence</th>
<th>Wasp species</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Polybia-MPI</em></td>
<td>IDWKKLLDAAKQIL</td>
<td><em>P. paulista</em></td>
</tr>
<tr>
<td>Mastoparan-A</td>
<td>IKWKALLDAVKKYVL</td>
<td>Vespa analis</td>
</tr>
<tr>
<td>Mastoparan-M</td>
<td>ILKAILALAKKLL</td>
<td>Vespa mandarina</td>
</tr>
<tr>
<td>Prototoxictina-MP</td>
<td>INWKALLDAKKVL</td>
<td><em>P. sylvestris</em></td>
</tr>
<tr>
<td>Mastoparan-C</td>
<td>INWKALLDAKKVL</td>
<td>Vespa crabro</td>
</tr>
<tr>
<td>Prototolypus-MPI</td>
<td>INWKALLDAAKVYL</td>
<td><em>P. exigua</em></td>
</tr>
<tr>
<td>Prototolypus-MPII</td>
<td>INWKAILAAKVQL</td>
<td><em>P. exigua</em></td>
</tr>
<tr>
<td>Apothea-MP</td>
<td>INWKALLDAAKQIL</td>
<td><em>A. p. pallipes</em></td>
</tr>
<tr>
<td>Prototolypus-MPIII</td>
<td>INWKALLDAAKQIL</td>
<td><em>P. exigua</em></td>
</tr>
</tbody>
</table>

Fig. 1. Chromatogram profile of fractionation of venom extract of the *Polybia paulista* venom under reverse-phase HPLC with a C-18 (ODS) CAPCELL PACK UG120 column (250 mm × 10 mm, 5 μm), under linear gradient from 5 to 60% (v/v) MeCN (containing 0.1% TFA), at a flow rate of 2.0 mL/min over 60 min by monitoring at UV 214 nm.

Fig. 2. (a) Chromatogram profile of re-fractionation of fraction 11 under reverse-phase HPLC with a C-18 (ODS) CAPCELL PACK UG120 column (250 mm × 10 mm, 5 μm), under isocratic condition (41% (v/v) MeCN containing 0.1% TFA), at a flow rate of 2.0 mL/min over 20 min by monitoring at UV 214 nm. (b) Chromatogram profile of re-purification of fraction 12 under reverse-phase HPLC with a C-18 (ODS) CAPCELL PACK UG20 column (250 mm × 10 mm, 5 μm), under isocratic condition (45% (v/v) MeCN containing 0.1% TFA), at a flow rate of 2.0 mL/min over 20 min by monitoring at UV 214 nm.
conservation in relation to the sequences of the mastoparan peptides, particularly in relation to Protonectarina-MP isolated from the venom of the tropical social wasp Protonec- tarina sylveirae, which has 71% of similarity in relation to the peptide component of fraction 11C. Thus, this peptide was classified as a mastoparan (MP) and was named Polybia-MPI. When the sequence of peptide component from fraction 12A is compared to the sequences available in the literature, as shown in Table 2, it is possible to observe 90% of similarity in relation to the chemotactic peptide previously reported as Protonectin in the venoms of two different species of neotropical social wasps, Protonectarina sylveirae [4] and A. p. pallipes [20]. Thus, the peptide isolated from fraction 12A will be referred as Polybia-CP.

### Table 2

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Primary sequence</th>
<th>Wasp species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paulista-CP</td>
<td>ILGTILGLLKSL</td>
<td>P. paulista</td>
</tr>
<tr>
<td>Protonectin</td>
<td>ILGTILGLLKGLL</td>
<td>P. sylveirae/p. pallipes</td>
</tr>
<tr>
<td>Ves-CP-T</td>
<td>FLPIGLKILGGLL</td>
<td>V. tropica</td>
</tr>
<tr>
<td>Ves-CP-M</td>
<td>FLPIJGLKLGLL</td>
<td>V. mandarina</td>
</tr>
<tr>
<td>Ves-CP-X</td>
<td>FLPIJAKLGLL</td>
<td>V. xanthoptera</td>
</tr>
<tr>
<td>Ves-CP-P</td>
<td>FLPIJAKLVSGLL</td>
<td>Pancoeoplia lewisi</td>
</tr>
</tbody>
</table>

#### 3.2. Biological activities

Biological activities of Polybia-MPI and Polybia-CP were investigated by assaying mast cell degranulation, release of LDH activity, hemolysis, chemotaxis and antimicrobial activities.

Fig. 3 shows the results of rat peritoneal mast cell degranulation for Polybia-MPI and Polybia-CP, revealing that both peptides presented a reduced activity at $10^{-5}$ M, while a reasonable activity was observed when the peptides concentration was $10^{-4}$ M (62 and 52% degranulation for Polybia-MPI and Polybia-CP, respectively); in fact, this concentration is too high to speculate about any important action under physiological conditions.

Fig. 4 shows the results of rat peritoneal mast cell lysis by LDH measurement in the medium. Polybia-MPI presented about 50% of LDH activity at $4.5 \times 10^{-5}$ M and Polybia-CP only reach this percentage at concentration higher than $10^{-4}$ M.

Hemolysis of rat erythrocytes was also examined for both peptides and the results are represented in Fig. 5. Polybia-MPI presented no hemolytic activity, while Polybia-CP presented a very reduced hemolysis at $10^{-5}$ M, but caused 78% hemolysis at the concentration of $10^{-4}$ M.

Polybia-MPI and Polybia-CP presented a very high chemotaxis of PMNL cells ($2.4 \times 10^4$ and $1.7 \times 10^4$ cells/ml, respectively) at the concentration of $10^{-4}$ M (Fig. 6).

The antimicrobial activity was examined and the results summarized in Table 3. Polybia-MPI presented a potent antimicrobial activity against both Gram-positive and Gram-negative bacteria, while Polybia-CP demonstrated a significant antimicrobial activity only against Gram-positive bacteria.

Fig. 3. Degranulation activity in rat peritoneal mast cell. The activity was determined by measuring the release of the granule marker, $\beta$-glucosaminidase, which co-localizes with histamine and the values for $\beta$-glucosaminidase released in the medium were expressed as the percent of total $\beta$-glucosaminidase. Values are mean ± S.D. (n = 5).

Fig. 4. LDH activity in rat peritoneal mast cell. The activity was determined by measuring the presence of the lactate dehydrogenase activity to the medium; the results were shown as relative activity by using the total LDH activity contents of rat mast cells lysed in presence of 0.1% (v/v) Triton X-100 (considered as 100%). Results are expressed as mean ± S.D. (n = 5).

Fig. 5. Hemolytic activity in washed rat red blood cells (WRBCC). The absorbance measured at 540 nm from lysed WRBCC in presence of 1% (v/v) Triton X-100 was considered as 100%. Values are mean ± S.D. (n = 5).
Most chemotactic peptides of wasp species endemic to cold regions of the planet present a characteristic FLP tripeptide at the amino terminal side, which is missing in Polybia-CP and Protonectin.

- A central cationic Lys residue is observed at the seventh position in the chemotactic peptides from cold climatic species, while Polybia-CP and Protonectin contain a cationic Lys residue at the 10th position.
- The only structural difference between Protonectin and Polybia-CP is the replacement of the Gly residue at the 11th position in the sequence of Protonectin by a Ser in Polybia-CP.

Delivery of stored compounds from mast cell granules may occur either due to the cytolytic effect of the peptides or due to exocytosis activated by the binding of the peptides to G-protein coupled receptors, which in turn activate a cascade of molecular events, resulting in mast cell degranulation [10]. Some mastoparan peptides seem to be involved in guanylate cyclase activation, either interacting directly with the enzyme or through other proteins [32].

Despite classification as a mastoparan peptide and to present a high similarity of primary sequence when compared to Protonectin-MP, the rat mast cell degranulation activity of Polybia-MPI is reduced (EC50 = 4.5 × 10−5 M) when compared to Protonectin-MP (EC50 = 4 × 10−6 M) [3]. Polybia-MPI caused the release of 62% of the contents from rat mast cells at 4.5 × 10−5 M (Fig. 3), however it also promoted the delivering of 50% of LDH activity from these cells at the same concentration range. Therefore, taking into account both results together and the standard deviation of both experiments, it may be concluded that the peptide caused the lysis of 50% of rat mast cells, which in turn led to the delivering the granules contents of these cells.

Polybia-MPI-induced no hemolytic suggesting that it demonstrates a poor interaction with the zwitterionic membranes of rat erythrocytes (Fig. 5), despite application of increasingly high doses in the assays. Thus, Polybia-MPI seems to be a much higher affinity to interact with the membranes of mast cells than of erythrocytes.

Polybia-CP presented both a reduced mast cell degranulation activity at 10−6 M (Fig. 3) and mast cell lysis (Fig. 4) as expected for the most of chemotactic peptides from wasp venoms [22]; this peptide also has a poor hemolytic activity in rat erythrocytes in the same concentration range (Fig. 5), as well as its natural analogue Protonectin [3,20]. The activities observed at the concentration of 10−4 M is too high to have any physiological meaning. Thus, the peptide Polybia-CP does not seem to interact with the zwitterionic membranes of rat erythrocytes and rat mast cells.

Chemotaxis is the phenomenon in which bacteria or single cells of multicellular organisms direct their movements according to the presence of specific chemical signals in their environment. The recruitment of leukocytes to a site of tissue injury caused by a wasp sting, constitutes a cause for inflammatory responses. Mechanistically, it involves a cascade of cellular events precisely regulated by temporal and spatial presentation of a repertoire of molecules in the migrating leukocytes and their surroundings (microenvironments) [16]. Eukaryotic cells in general sense the presence of...
chemotactic stimuli through stereospecific 7-transmembrane heterotrimeric G-protein coupled receptors; the activity of the chemotactic peptides generally is dependent on specific signals mediated by G-proteins located on the plasma membrane of the chemotactically cells, making the cell/peptide interaction relatively selective [22]. Although the mechanisms of induction of PMNL cells attraction by the peptides Polybia-MPI and Polybia-CP is not known, these peptides probably elicit their effect by interacting with G-proteins at the level of the PMNL cell membrane, as previously reported for other chemotactic peptides from the venom of the tropical social wasp *P. exiguus* [21].

Generally, the peptide wasp toxins are polycationic peptides which may adopt a α-helix conformation and present amphiphilic properties, which are essential to exhibit their biological activities [30]. Thus, they can interact with the anionic components of the bacterial membranes in different ways, sometimes resulting in irreversible damage to the cell. Among the known toxins from wasps venom the peptides Crabrolin [15], Anoplin [14] and Protonectin [20] were also reported to present antimicrobial activity.

Polybia-MPI presented antimicrobial activity against both Gram-positive and Gram-negative bacteria, while Polybia-CP presented antimicrobial action only against the Gram-positive bacteria. In order to explain these effects based on the possible interactions of each peptide with the bacterial membranes, the secondary structures of Polybia-MPI and Polybia-CP were predicted by using the software “Consensus Secondary Structure Prediction”, from the Network Protein Sequence Analysis (http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=NPSA/npsa_seccons.html). The algorithm predicted that Polybia-MPI has about 71.43% of α-helix conformation and 28.57% of coil structure, while Polybia-CP was predicted to present 50% of random coil and 50% of ambiguous conformations. Thus, it seems clear that the high content of α-helix of Polybia-MPI must aid its assembly in the bacterial membrane, reflecting the lower MIC values observed for this peptide, when compared to Polybia-CP.

The cell wall is a complex structure, fundamentally different in Gram-positive and Gram-negative bacteria. The cell wall of bacteria consists of a polymer of disaccharides cross-linked by short chain peptides, forming a type of peptidoglycan called murein. In the Gram-positive bacteria, the cell wall is thick (15–80 nm), consisting of several layers of peptidoglycan complexed with molecules of teichoic acids. In the Gram-negative bacteria, the cell wall is relatively thin (10 nm) and composed of a single layer of peptidoglycan surrounded by a membranous structure, the outer membrane, which invariably also contains lipopolysaccharides. Thus, the outer membrane is more hydrophobic in the Gram-negative than in the Gram-positive bacteria and constitutes the target for attack by hydrophobic agents and other antibacterial agents [29,31].

According to Mendes et al. [19] Protonectin has antimicrobial action against both Gram-positive and Gram-negative bacteria, while the peptide Polybia-CP was effective only against Gram-positive bacteria. The difference between the sequences of both peptides is the replacement of Gly residue at the 11th of Protonectin, by a Ser residue in Polybia-CP. The side chain for the Gly residue is hydrogen, while for the Ser residue is a hydroxy methyl group. Thus, the C-terminal region of Polybia-CP is more hydrophobic than the corresponding region of Protonectin, which could at first, explains the better interaction of Protonectin with the more hydrophobic characteristics of the outer membrane of the Gram-negative bacteria.

Thus, the present investigation describes the isolation, purification, sequencing and biological characterization of two novel biologically active peptide toxins from the venom of the tropical social wasp *P. paulista*. These toxins constitute members of polycationic, linear peptides deprived of cysteine residues, and demonstrating multifunctional activities such as: mast cell degranulation, chemotaxis of PMNL cells, cytolyis of erythrocytes and antimicrobial activity.

Acknowledgements

This work was supported by a grant from the São Paulo State Research Foundation (FAPESP); M.A.M is Postdoctoral fellow from FAPESP (Proc. 01/55060-4), B.M.S. and M.R.M are Doctoral students fellows from FAPESP; L.D.S. and L.M.M.C are Doctoral student fellow from CAPES. Mario Sergio Palma (Proc. 300377/2003-5) and Fernando Carlos Pagnocca are researching for the Brazilian Council for Scientific and Technological Development (CNPq).

References


