De novo sequencing of antimicrobial peptides isolated from the venom glands of the wolf spider Lycosa singoriensis

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Antimicrobial peptides (AMPs), named lycocitin 1, 2 and 3, and a peptide with a monoisotopic molecular mass of 3038.70 Da were detected in the venom glands of the wolf spider Lycosa singoriensis. Two of the peptides, lycocitin 1 and 2, are new AMPs whereas lycocitin 3 is highly homologous to lycotoxin II isolated from the venom of spider Lycosa carolinensis. In addition, two other peptides with monoisotopic masses of 2034.20 and 2340.28 Da showing the motif typical for antimicrobial peptides were also identified. These peptides and lycocitin 1, 2 and 3 were de novo sequenced using electron capture dissociation and low-energy collisional tandem mass spectrometry. The amino acid sequence of lycocitin 1 was determined as GKLQAFLAKMKEIAAQTL-NH2. Lycocitin 2 differs from lycocitin 1 by a replacement of a lysine residue for an arginine residue at the second position. Lycocitin 3 differs from the known lycotoxin II consisting of 27 amino acid residues by a deletion of Gly-26. Both lycocitin 1 and 2 inhibit growth of Gram-positive (Staphylococcus aureus, Bacillus subtilis) and Gram-negative (Escherichia coli) bacteria and fungi (Candida albicans, Pseudomonas aeruginosa) at micromolar concentrations.

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INTRODUCTION

Antimicrobial peptides (AMPs) are the key elements of the innate immunity against bacteria and fungi in both the animal and plant kingdoms (for reviews, see Refs 1–6). Over 800 AMPs have already been described (http://bbcm1.univ.trieste.it/~tossi/pag1.htm). Natural animal venoms are good sources of potential antimicrobial substances, venoms of Arthropoda being of special interest. More than 40 000 species of poisonous arthropods are currently known, in which spiders and scorpions are the main species. Their venoms contain a large number of diverse biologically active components of various chemical structures. However, not more than 150 arthropod species have been investigated so far and only few of them have been characterized for the presence of antimicrobial activity. Two types of linear cationic AMPs consisting of 13–27 amino acid residues were found in arthropod venoms: cecropin-like and mellitin-like types.7 The AMPs of the first type are mainly detected in the hemolymph of various arthropods such as sacrotoxin in house fly,8 cecropins in lepidoptera9 and spinegirin in termites.10 AMPs of the second type were found in the venom glands of bees,11 wasps,12 ants,13 scorpions14 and spiders such as Lycosa carolinensis,15 Cupiennius salei16 and Oxyopes kitabensis.17 The composition of tarantula spider venom in relation to sex, age and geographical origin was studied by Escoubas and co-authors using high-performance liquid chromatography (HPLC) and capillary electrophoresis in combination with different mass spectrometric methods.18–21

In this paper, we describe the isolation, structural evaluation and biological activity of lycocitin 1, 2 and 3 and two peptides that are possible candidates for AMPs from the venom glands of the wolf spider Lycosa singoriensis.
EXPERIMENTAL

Chemicals
Acetonitrile (ACN), trifluoroacetic acid (TFA), all salts, α-cyano-4-hydroxycinnamic acid (CHCA) and 2,5-dihydrobenzoic acid (DBH) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Pipette tips for sample preparation (C18, ZipTip) were obtained from Millipore (Bedford, MA, USA) and reversed-phase (RP) packing medium (POROS 10R2) was obtained from PerSeptive Biosystems (Framingham, MA, USA); GE Loader tips were purchased from Eppendorf (Hamburg, Germany).

Isolation of lycocitins
Wolf spiders, Lycosa singoriensis (Aranae: Lycosidae), were collected from the Fergana region of Uzbekistan, Central Asia. The venom glands were dissected and homogenized in water (1:10, w/v, 2×) for 30 min at 4 °C. The combined extract was centrifuged at 14000 × g for 30 min and the supernatant was lyophilized. A 2 mg amount of the lyophilized extract was centrifuged at 14000 × g for 30 min and the supernatant was lyophilized. A 2 mg amount of the lyophilized extract was dissolved in 100 µL of the elution buffer (20 mM phosphate buffer, pH 4.5, 150 mM NaCl) and injected onto a TSK 2000 SW column (250 × 4.6 mm i.d.) using a linear ACN gradient (10–40% B in 30 min) and then eluted with an isocratic gradient (40% B for 30 min) at a flow-rate of 0.1 mL min⁻¹. The active fractions were collected on a SpeedVac.

Mass spectrometry
Sample preparation for matrix-assisted laser desorption/ionization (MALDI) MS
Approximately 1 µg of the crude lyophilized extract from the venom glands dissolved in 10 µL of 0.1% TFA was concentrated and desalted on a C18 ZipTip according to the manufacturer’s protocol. The peptides were eluted with 2.5 µL of MALDI matrix solution (saturated solution of CHCA in ACN–0.1% TFA (45:55, v/v)). The first 1–5 droplets were deposited directly on a target plate. Mass spectra were acquired on an Voyager-Elite MALDI time-of-flight (TOF) mass spectrometer (PerSeptive Biosystems). Spectra were recorded in the positive ion linear mode using an acceleration voltage of 20 kV. On average, 256 pulses were obtained for each mass spectrum. Calibration of the spectrometer was performed with a mixture of insulin β-chain and bovine insulin. The molecular masses of purified lycocitins were determined by MALDI fourier transform ion cyclotron resonance (FTICR) MS with an internal calibration peak from ACTH 1–24 peptide using DHB as a matrix.

Sample preparation for nano-electrospray ionization (ESI) MS
Peptides were desalted and concentrated on a nano-column of 10 POROS R2 material packed into GE Loader tips as described previously. The peptide was eluted from the column using 2 × 1 µL of ESI solvent (MeOH–H₂O–HCOOH (50:45:5)) directly into a metal-coated nano-electrospray needle (Protana Engineering, Odense, Denmark), and the fraction was subjected to MS analysis.

Tandem mass spectrometry (MS/MS)
All electron capture dissociation (ECD) fragmentation spectra were acquired on an Ultima Fourier transform mass spectrometer (IonSpec, Irvine, CA, USA) equipped with a 4.7 magnet, a custom-built nano-electrospray ion source and a standard hexapole-based interface (Analytica of Branford, Branford, MA, USA). The ions were externally accumulated in the hexapole for 600 ms, then transported to the infinity cell, where they were captured in a gated mode without a cooling gas. The precursor ions for MS/MS were isolated by on-resonance ejection of undesired ions. In ECD experiments, precursor ions were irradiated for 700 ms with subthermal electrons (<0.2 eV) produced by a standard EI tungsten-filament installed behind the FTICR cell and operated using accelerated voltages ranging from +0.75 V to −1.25 V, in order to produce the low-energy electrons.

Collision-induced dissociation (CID) MS/MS experiments were performed on both the above Ultima instrument and on a QSTAR Pulsar hybrid quadrupole time-of-flight (qTOF) tandem mass spectrometer (AB/MDS-Sciex, Toronto, Canada) with pulsed Q2 fragment ion injection. The latter instrument was equipped with a nano-electrospray ion source (Protana Engineering). The total duration of a single experiment was 2 s in ECD-MS 5 s in CID FTICR-MS and 10 s in CID qTOF experiments, which allowed for accumulation of multiple scans within a several-minute run. The samples were sprayed at a flow-rate of ~1 µL h⁻¹ when applying a needle voltage of 700 V. A survey mass spectrum and a tandem mass spectrum were recorded for each sample. During MS/MS, the fragment ions were generated from the isolated 5+ charged peptide precursor ion and fragmented by low-energy CID. The CID-MS/MS fragmentation was carried out using a collision energy of 45 V. Nitrogen gas was introduced into the collision cell Q2 to a pressure of 4 mTorr. The CID experiments were carried out using an FTICR mass spectrometer with nitrogen (pulse duration 2 ms) as target gas and r.f. excitation of analyte ions in the cell. The amino acid sequences were identified from the ECD and CID tandem mass spectra of peptides using Inspector sequence tag prediction software.

N-Terminal sequencing
The N-terminal amino acid sequences of lycocitins were determined on a Model 492 Procise protein/peptide sequencer (Applied Biosystems, Palo Alto, CA, USA) according to the manufacturer’s protocol.

Peptide synthesis
Synthetic lycocitin 1 and 2 were obtained on an Intavis (Cologne, Germany) automated peptide synthesizer. The peptides were prepared by solid-phase synthesis on TentaGel
RRAM universal amide resin, capacity 0.19 mmol g⁻¹ (RAPP Polymere, Tübingen, Germany) using Fmoc chemistry. The peptide cleavages from the resin and side-chain deprotection were achieved using TFA, thioanisole and ethanethiol. Cleavage was conducted for 3 h at room temperature, followed by precipitation of the peptide using methyl tret-butyl ether. The peptides were lyophilized and purified by RP-HPLC on a Nucleosil-100 C18 column (250 × 10 mm i.d.) at a flow-rate of 1.5 ml min⁻¹ and 40 °C using an ACN gradient (10–40% B in 60 min, 40% B for 20 min). The purity and identity of synthetic peptides as lycocitin 1, 2 and 3 were confirmed by RP-HPLC, MS, amino acid analysis and N-terminal sequencing (not shown).

Antimicrobial assays

HPLC fractions were tested for antimicrobial activity against *E. coli* ATCC25922 and lycocitin 1 and lycocitin 2 were tested for antimicrobial activity against Gram-negative bacteria (*E. coli* ATCC25922, *Pseudomonas aeruginosa* ATCC27853), Gram-positive bacteria (*Bacillus subtilis* ATCC6633) and fungi (*Candida albicans*) by a liquid growth inhibition assay. Bacteria were grown in liquid broth (LB) (1% Bacto-Tryptone, 0.5% Bacto yeast extract, 1% NaCl in 10 mM Tris, pH 7.6) at 37 °C for 15 h. Then, 100 µl of the cell culture were added to 5 ml of the medium and bacteria were grown under the same conditions as above (D₅₀₀ = 0.5). Biological activities of HPLC samples were determined against *E. coli* ATCC25922 in 96-well sterile plates in a final volume of 100 µl as follows. Dried HPLC samples redissolved in 10 µl of water were added to 90 µl of LB containing the inoculate of *E. coli* ATCC25922 adjusted to 5 × 10⁸ colony-forming units/ml. After 15 h of incubation at 37 °C, growth inhibition was determined by measuring the absorbance at 620 nm. Minimal inhibitory concentrations (MICs) of synthetic lycocitin 1 and 2 were determined against *E. coli* ATCC25922 and *Bacillus subtilis* ATCC6633 in the range 0.4–50 µl of 10 µl of different concentrations of peptides dissolved in water were added to 90 µl of LB broth containing the inoculate of *E. coli* ATCC25922 or *Bacillus subtilis* ATCC6633, adjusted to 5 × 10⁸ colony-forming units/ml. MIC was determined as the highest concentration that resulted in significant differences in cell growth compared to the control. The purity and identity of synthetic peptides as lycocitin 1, 2 and 3 were confirmed by RP-HPLC, MS, amino acid analysis and N-terminal sequencing (not shown).

RESULTS AND DISCUSSION

Isolation of lycocitins

The extract of venom glands contains at least 50 components, which can be detected by RP-HPLC (not shown) with molecular masses (averaged) determined by MALDI-TOF-MS: 1960.49, 1988.86, 3038.50, 3086.12, 3149.75, 3627.34, 3816.6, 4411.15, 4730.55, 4874.24, 5019.08, 5161.58, 5781.60, 5811.89, 7255.89, 7388.20, 7662.28, 7973.59 Da (only significant peaks in the mass spectra were evaluated). Therefore, the total extract was pre-separated by size-exclusion (SE) HPLC and active fractions C and D were further separated by RP-HPLC (Fig. 1). As a result, three AMPs with molecular masses 1960.49, 1988.86 and 3149.75 Da named lycocitin 1, lycocitin 2 and lycocitin 3, respectively, were isolated. In addition, one active 3038.70 Da peptide was detected (it was not studied further). Two peptides with monoisotopic masses of 2034.20 and 2340.28 Da were detected by ESI-TOF-MS in fraction D. They both co-eluted with lycocitin 3 during RP-HPLC separation (Fig. 1(C)). Lycocitin 3 was found in two fractions, C and D. It is of particular interest that the peptides with masses below 3 kDa were not detected earlier in the venom of different spider species. This might be explained by the method of peptide isolation used in this study. We extracted peptides from the homogenized venom glands whereas AMPs were typically isolated from venom.

Structures of lycocitins, 2034 and 2340 Da peptides

In this study, we combined the ECD, CID and ultra-high-resolution capabilities of the FTICR mass spectrometer with the CID-MS/MS and the high-resolution capabilities of the hybrid quadrupole TOF mass spectrometer to identify the *de novo* amino acid sequence of the antimicrobial peptides isolated from the venom glands of the wolf spider *Lycosa singoriensis*. Peptide amino acid sequences determined by ECD produced c and z fragment ions that give the whole amino acid sequence, which can be confirmed by CID-MS/MS that generated b and y type fragment ions. ECD used in conjunction with low-energy CID by the ‘golden pair’ approach is a comprehensive tool for *de novo* sequencing of an unknown peptides.

**Lycocitin 3**

The ECD spectrum of the quintuply charged ion MH⁺⁵⁺ is shown in Fig. 2. The electrosprayed ions were externally accumulated for 600 ms in a hexapole and subsequently ejected into the ICR cell as a package. In the ICR cell ions of interest were isolated by the SWIFT technique. A tungsten filament was used to produce low-energy electrons to irradiate trapped ions for 700 ms. All fragment ions and the precursor ion and reduced species were detected simultaneously by a standard procedure. Two hundred scans were signal averaged. The spectrum obtained was very informative, but interpretation was difficult, since it contained more than 10 isotopic peak clusters. The spectrum was processed using Inspector software (MDS Inc, Odense, Denmark) assuming that ECD gives c and z ions. The nearly complete sequence of a 26-residue long peptide was obtained. The identified fragments contained...
Figure 1. Isolation of lycocitins from the venom glands of the spider Lycosa singoriensis. (A), Separation of 2 mg of the total extract by SE-HPLC on a TSK2000SW column; (B) and (C), separation of active fractions C and D by RP-HPLC, respectively. Arrows indicate the elution position of active peptides with their molecular masses (averaged): 3149.50 (lycocitin 3), 3038.70 (unknown, not studied), 1960.30 (lycocitin 1) and 1988.40 (lycocitin 2). 2034.20 and 2340.28 Da peptides were both detected in the SE-HPLC fraction D and co-eluted with lycocitin 3. The dashed lines show acetonitrile gradients.

22 singly charged \( c_{16}^+ \) and \( z_{12}^+ \) fragments, 32 doubly charged \( c_{23}^{2+} \) and \( z_{15}^{2+} \) fragments and 17 triply charged \( c_{16}^{3+} \) and \( z_{23}^{3+} \) ions. A single unassigned gap between residues 23 and 25 in the sequence, with a mass of 170.1 Da, was found. This mass fitted well with the mass of the amino acid pairs Ala–Val (170.105 Da) and Leu/Ile–Gly (170.105 Da). To verify the complete sequence and especially the gap pair residues, a multiple storage assisted dissociation (MSAD)\(^{26}\) CID-MS/MS experiment was performed. Using the MSAD technique, the lycocitin 3 peptide was fragmented in the hexapole of FTICR instrument. The \( y_{17} \) fragment was isolated and subjected to CAD with a 2 ms \( N_2 \) gas pulse in the analyser cell. The CAD fragmentation spectrum of the \( y_{17} \) ion contained a cleavage between the 15th and 16th amino acid residues, giving \( b_{15}^{2+} \) ion with a mass \( m/z \) 878.174 that indicates a Leu/Ile–Gly amino acid pair in the sequence (Fig. 3). As a result of these two experiments, the complete sequence of lycocitin 3 was obtained except for distinction between Leu and Ile. A fragmentation technique called Hot ECD (HECD)\(^{27,28}\) that allows for distinction of Leu and Ile amino acid residues was later developed in our laboratory.

The QSTAR nanoESI-MS survey scan spectra of lycocitin 3 give weak singly, doubly and triply-charged molecular ions, but pronounced quadruply and quintuply charged
molecular ions. The quintuply charged ion MH$_5$$^+$ at m/z 636.96 of lycocitin 3 was selected with a mass isolation window wide enough to include the entire isotope cluster. The neutral mass for this peptide ion is 32 Da higher than the corresponding ion observed in the FTICR mass spectrum; this strongly indicates in-solution oxidation of two methionine (Met) groups, which is known to occur spontaneously.$^2^9$

The neutral mass for this peptide ion is 32 Da higher than the corresponding ion observed in the FTICR mass spectrum; this strongly indicates in-solution oxidation of two methionine (Met) groups, which is known to occur spontaneously.$^2^9$

The QSTAR fragment ion spectrum is highly complicated with more than 200 fragment isotope clusters identified in the mass range m/z 100–1000. In the low-mass region of the spectrum, immonium and terminal sequence ions indicated the possibility of several amino acid residues being present in the peptide (Fig. 4), and at the same time excluded other amino acid residues. Abundant low-mass peaks at m/z 110, 120 and 159 indicate the presence of histidine, phenylalanine and tryptophan, respectively. To assign other fragment ions and to determine the amino acid sequence, the tandem mass spectra were analysed using the Inspector software. It was possible to assign the complete y ion series from y$_{25}$$^+$ to y$_1$$^+$ by a stepwise generation of the sequence (MH$_5$$^+$ − y$_{25}$$^+$, y$_{24}$$^+$ − y$_{23}$$^+$, y$_{22}$$^+$ − y$_{21}$$^+$, y$_{20}$$^+$ − y$_{19}$$^+$, y$_{18}$$^+$ − y$_{17}$$^+$, y$_{16}$$^+$ − y$_{15}$$^+$). The y ions were very abundant in the CID spectrum shown in Fig. 5.

Using the Inspector software, it was also possible to assign a complete sequence of b ions from b$_1$$^+$ to b$_{25}$$^+$ (MH$_7$$^+$ − H$_2$O); b$_1$$^+$ − b$_{10}$$^+$, b$_{11}$$^+$ − b$_{14}$$^+$, b$_{15}$$^+$ − b$_{20}$$^+$, b$_{21}$$^+$ − b$_{25}$$^+$. The complexity of the spectra originates from characteristic neutral losses of water (−18 Da) and methanesulfenic acid (−64 Da) from b and y sequence ions. The facile loss of a methanesulfenic acid (CH$_3$SOH, −64 Da)$^{3^0}$ from b and y fragment ions was observed as prominent satellite peaks for each of the fragment ions containing an oxidized methionine (Mox) residue (Fig. 5).

Because the nominal residue mass of Mox (147.035 Da) is close to that of phenylalanine (F, 147.068 Da), the b − 64 and
y $- 64$ satellite ion series can be used to discriminate between the M$_{ox}$ and F residues as well as additional conformation information on the amino acid sequence. This allows for unambiguous determination of the positions of the two methionines and two phenylalanines, which is supported by data from the corresponding ECD spectrum. The QSTAR-CID spectrum confirms the amino acid sequence predicted from the ECD spectrum; all amino acid residues in the 26-residue long peptide were uniquely assigned except for differentiation between the isomeric residues leucine and isoleucine.

Lycocitins 1 and 2
The monoisotopic masses of lycocitin 1 and 2 were determined by MALDI-FTICR-MS, using ACTH 1–17 peptide as an internal calibration peak, are 1959.13 and 1987.15 Da, respectively. In the ESI source, both peptides have an abundant 3+ molecular ion peak. The triply charged species of lycocitin 1 and 2 were accumulated in the hexapole for 500 ms and then ejected into the cell. Subsequently they were isolated and irradiated by a 500 ms pulse of low-energy electrons. The ECD spectrum of lycocitin 1, shown in Fig. 6, demonstrates the full sequence coverage by c and z fragments. The ECD spectrum of lycocitin 2 (Figure 7) also contains the full sequence coverage of c and z fragments. Both peptides were C-terminally amidated and are identical except for the second amino acid residue: Lys in lycocitin 1 and Arg in lycocitin 2.

The structures of all three lycocitins (Table 1) are in good agreement with their amino acid compositions (not shown) and measured masses. The Ile/Leu assignment and the amino acid sequences of lycocitins were identified by the Edman degradation technique.

2034 and 2340 Da peptides
These low-abundance peptides were not isolated in this study. They have been detected (Fig. 1(C)) and de novo sequenced by MS methods described above (data not shown). Both peptides are linear, positively charged with high pI values, and their amino acid sequences seem to show a motif similar to that of the linear AMPs. The 2340 Da peptide is C-terminally amidated, whereas the 2034 Da peptide is not (Table 1).
Table 1. Antimicrobial peptides from the venom glands of the spider *Lycosa singoriensis*

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Amino acid sequence (^a)</th>
<th>Masses ((M_0)), measured (^d) (Da)</th>
<th>Masses ((M_0)), calculated (Da)</th>
<th>Mass difference (Da)</th>
<th>pI</th>
<th>Net charge at pH 7.0</th>
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<tr>
<td>Lycocitin 3(^b)</td>
<td>KIKWFKTMKSLAKFLAKEQMKKHGE-OH (26)</td>
<td>3147.63</td>
<td>3147.82</td>
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<td>10.80</td>
<td>7.0</td>
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<tr>
<td>Lycocitin 2(^b)</td>
<td>GRLQAFLKMKEIAAQT-NH(_2) (18)</td>
<td>1987.15</td>
<td>1987.14</td>
<td>0.01</td>
<td>10.61</td>
<td>2.0</td>
</tr>
<tr>
<td>Lycocitin 1(^b)</td>
<td>GKLQAFLKMKEIAAQT-NH(_2) (18)</td>
<td>1959.12</td>
<td>1959.13</td>
<td>−0.01</td>
<td>10.34</td>
<td>2.0</td>
</tr>
<tr>
<td>3038(^d)</td>
<td>n.d.</td>
<td>3038.70</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2034(^c)</td>
<td>AGIGKIGDFIKKAIKYKN-OH (19)</td>
<td>2034.21</td>
<td>2034.20</td>
<td>0.01</td>
<td>10.50</td>
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<tr>
<td>2340(^c)</td>
<td>MIASHLAEKSLGKSHTLM(_2)-NH(_2) (21)</td>
<td>2372.28</td>
<td>2340.28</td>
<td>32.0(^c)</td>
<td>10.26</td>
<td>3.1</td>
</tr>
</tbody>
</table>

\(^a\) Number of amino acid residues is given in parentheses.

\(^b\) Leu/Ile was assigned by a chemical degradation method.

\(^c\) Leu/Ile was not assigned.

\(^d\) The molecular masses of lycocitins were determined by MALDI-FTICR-MS and those of the 3038 Da peptide by MALDI-TOF-MS and 2034 and 2340 Da peptides by ESI-TOF-MS.

**Figure 6.** ECD spectrum of \([M + 3H]^{3+}\) of lycocitin 1, 100 scans.

**Figure 7.** ECD spectrum of \([M + 3H]^{3+}\) of lycocitin 2, 100 scans.
Structural features and antimicrobial activity of lyocitins

Lyocitin 1 and 2 are linear peptides consisting of 18 amino acid residues; both are C-terminally amidated and positively charged (Table 1). Lyocitin 1 and 2 differ only at the second position of the peptide, with Lys and Arg, respectively. The amino acid sequences of lyocitin 1 and 2 are characterized by a cluster distribution of hydrophobic and charged amino acid residues: the Lys-9 and Lys-11 separated by Met-10 are flanked by hydrophobic amino acid residues except for Lys-2/Arg-2. They have unique amino acid sequences and are the smallest molecular mass peptides found in spider venoms. Lyocitin 3 is also a typical linear amphipathic peptide consisting of 26 amino acid residues. Its net charge at physiological pH is 7+. Lyocitin 3 is identical with lytocotxin II isolated from the venom of the spider Lycosa carolinensis with the exception of a Gly-26 deletion and homologous to cecropin-type AMPs, with typical repeats of lysine residues. Our data show that the lyocitin 2 demonstrates a broad spectrum of antimicrobial activity against Gram-negative bacteria (E. coli ATCC25922, Pseudomonas aeruginosa ATCC27883), Gram-positive bacteria (Bacillus subtilis ATCC6633 and ATCC6533, Micrococcus luteus, Staphylococcus aureus 209P) and fungi (Candida albicans) in plate growth inhibition assays. The MICs of lyocitin 1 and 2 were measured in liquid-phase assays in the range 0.4–50 µM. The MICs of lyocitin 2 against E. coli ATCC25922, Pseudomonas aeruginosa ATCC27853 and Bacillus subtilis ATCC6633 were 3.14–6.29, 5.02–10.05 and 1.57–3.14 µM, respectively, and against Candida albicans 1.25–2.51 µM. The MICs of lyocitin 1 against E. coli ATCC25922 and Bacillus subtilis ATCC6633 were 3.2–6.4 and 1.6–3.2 µM, respectively. These data show that the MICs of the lyocitins 1 and 2 are similar. Since the amino acid sequence of lyocitin 3 differs from that of the known lycotoxin II by only one deletion, its biological activity was not studied.

CONCLUSION

All three peptides, lyocitin 1, 2 and 3, display a broad spectrum of antimicrobial activities against Gram-positive and Gram-negative bacteria and fungi and were isolated from the venom glands of the spider Lycosa singoriensis. Two of them, lyocitin 1 and 2, are newly described AMPs. In this study, ECD and low-energy CID-MS/MS together with MSAD techniques were used for de novo amino acid sequencing of these lycocitins and of the low-abundance 2034 and 2340 Da peptides. These methods provided all necessary sequence data except for the elucidation of Ile/Leu. The HECQ technique for distinguishing between leucine and isoleucine amino acid residues, in conjunction with our described approach for sequencing, demonstrates the possibility of sequencing unknown peptides by MS at a sensitivity level that cannot be achieved by any other technique so far.

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