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Isolation and Characterization of an Antimicrobial Polypeptide from Loach

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Abstract: A novel antimicrobial polypeptide was isolated and characterized from loach, *Misgurnus anguillicaudatus*. The polypeptide, named MAPP, is a single-chain polypeptide with Mw about 9,800 Dalton and pI about 4.78; the N-tag of MAPP was CFGWN. MAPP showed good inhibition against various bacteria including *Bacillus subtilis, Escherichia coli and Staphylococcus aureus*. MAPP could be used as a lead compound in antibiotics drug discovery.

Key words: *Misgurnus anguillicaudatus*, isolation, characterization.

Hydrobionts formed their special defense systems during evolution. One such system is that of non-specific immunity which comprises a wide variety of peptides with potent antimicrobial activities[1]. The mechanism of action of most antimicrobial peptides was reported as that a few peptide molecules formed a channel on cell membrane, and the cell was then died of the outflowing of cellular contents. The above mechanism was different from that of antibiotics[2, 3]. It is a promising area to discover new antimicrobial peptides given the strongly increased drug-resistance of pathogens, and that the new antibiotics are harder to find[4]. Here we report a novel antimicrobial peptide (MAPP) from loach. Its isolation, characterization and bioactivities were also described in this paper.

Materials

All reagents for electrophoresis were purchased from Sigma; other chemicals were all at the grade of A.R; Bacteria were purchased from "China Centre for Type Culture Collection".

MAPP purification

Loaches were collected from southern china and raised in clean water for 4 days. Healthy loaches (700 g) were homogenated with 1,000 mL buffer (0.05 M Tris-Cl, pH 8.0, pepstatin A at 1 μg/mL) and the homogenate was lyophilized immediately. The solid matter was subsequently extracted with 1,000 mL 0.1 M Tris-Cl buffer (1.0 % Triton X-100, Pepstatin A at 0.1μg/ml, pH 8.15) for 12 h. Then the extracted supernatant was

acetone-graded to collect 3V-5V fractions. The collection was dialyzed (SnakeSkin ™ 3,500 Dalton cut off, Pierce) against 40 mM Tris-Cl before lyophilized and the obtained protein sample was uploaded on Sephadex G-50 column (2.6 cm×150 cm, Pharmacia) and DE-52 column (2.6 cm×25 cm, Whatman, with 0.05-0.5 N NaCl gradient employed in elution) to collect active peaks. 0.05 M Tris-Cl Buffer was used for elution. The activity was detected by antimicrobial assay against *Bacillus subtilis*[5] and, the protein was quantified by previously reported methods[6]. The obtained protein sample was then separated by polyacrylamid gel electrophoresis system[7] and the band containing MAPP in slab gel was excised out for electroelution[8]. As shown in **Table 1**, about 0.2 mg white powder that was designated as MAPP was eventually obtained from 700 g loach.

MAPP characterization

Firstly the Mw value of MAPP was measured respectively to be 9,600 and 9,800 by using non-denaturing Sephadex G-50 gel filtration and denaturing Tricine SDS-PAGE[9, 10], which demonstrated that MAPP is a single-chain polypeptide. Secondly, the pI (isoelectric point) value was measured as 4.78 by using isoelectric focusing (IEF)[11]. Later on, the N-terminal sequence tag was identified to be CFFGWN using ABI protein sequencer, so far no protein was found to match this tag in protein databases around the world. The MS-fingerprint (**Figure 1**) and the amino acid (AA) composition (**Table 2**) was also obtained respectively through Bruker Reflex III MALDI-TOF-MS and Hitachi 835-50 amino acid analysis instrument. AA composition analysis revealed that MAPP was a polypeptide with 94 AA residues, which contained about l0 types of AA residue. Cysteine was the most abundant (approximately 20.2 mol %) in MAPP (**Table 2**).

Antimicrobial assessment

Plate-antimicrobial assessment[5] revealed that MAPP could kill bacteria including *Bacillus subtilis*, *Escherichia coli* and *staphylococcus aureus*. No inhibition was found against mould and yeast. Thermal stability assessment against *Bacillus Subtilis*[5] showed that MAPP is thermal stable and more than 70% of activity of MAPP against *Bacillus subtilis* could be possessed even when treated at 60°C for 30 minutes.

Table 1 The purification processes of MAPP

Purification Steps	Total Protein /g	MAPP /g	Purification Fold
Extraction Supernatant	8.6390	0.0128	.....
Acetone Precipitation	0.5811	0.0101	11.7
Sephadex G-50 Gel Filtration	0.1021	0.0068	3.83
DE-52 Cellulose Chromatography	0.0049	0.0028	8.57
Electrophoresis and Electroelution	0.0002	0.0002	1.75

Figure 1 The MS-fingerprint of MAPP

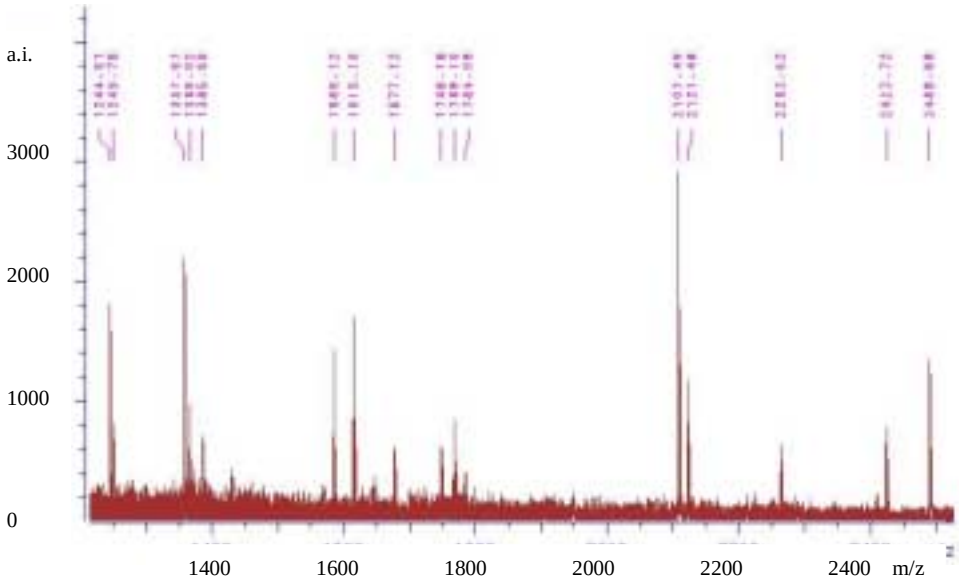

Table 2 The AA Composition of MAPP

AA	Residue number	Abundance	AA	Residue number	Abundance
CYS	19	20.2	ALA	8	8.5
GLY	17	18	SER	6	6.3
MET	11	11.7	GLU	6	6.3
VAL	9	9.5	LEU	5	5.3
TYR	9	9.5	ASP	4	4.2

Discussion

While MAPP showed a better inhibition of bacteria, it is very different from the known antimicrobial peptides. Generally, antimicrobial peptides are rich in alkaline residues that are positively charged in physiological conditions. Those peptides could be easily attracted by negative charged cellular membrane and they could consequently assemble a channel in the cell membrane. The cell was then killed by the outflow of cellular contents[1-3]. MAPP, on the other hand, didn't have any alkaline residue (but contains 19 cysteine residues) in the amino acid composition. Thus MAPP should have an unusual amino acid sequence that is related to the antimicrobial bioactivity that functions by a different mechanism compared with previously reported antimicrobial peptides. As a novel antimicrobial polypeptide, MAPP has a potential value to be used as lead compound in antibiotics drug discovery.

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