We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Determination of Staphylococcal Phenol-Soluble Modulins (PSMs) by a High-Resolution HPLC-QTOF System

Xiaoxiao Wu and Chi Zhang

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/66690>

Abstract

Phenol-soluble modulins (PSMs) are multifunctional, amphipathic, α -helical peptides produced by virtually all staphylococcal strains. They have recently drawn much attention owing to the key contribution of some PSM peptides to staphylococcal virulence, in particular in highly virulent *Staphylococcus aureus*. High concentration of PSMs may cause cytolysis, damaging neutrophils, immune cells, red blood cells and white blood cells. Furthermore, all PSMs contribute to biofilm structuring and the dissemination of biofilm-associated infection. Here we describe a method for PSM analysis in *S. aureus* by high-performance liquid chromatography-quadrupole time-of-flight mass spectrometry system (HPLC-QTOF).

Keywords: phenol-soluble modulins, HPLC-QTOF, *Staphylococcus aureus*, determination

1. Introduction

Phenol-soluble modulins (PSMs) are a family of multifunctional, amphipathic and α -helical peptides produced by virtually all staphylococcal strains [1–3]. They have recently drawn much attention owing to the key contribution of some PSM peptides to staphylococcal virulence, in particular in highly virulent *Staphylococcus aureus* [4]. In addition, they have antibacterial activity, likely to compete with other environmental rival bacteria such as streptococci [5, 6], as well as biofilm structuring and dissemination functions [7, 8]. *Staphylococcus aureus* is an important and versatile opportunistic human pathogen that can cause a wide range of acute and chronic diseases, which range from superficial infections to invasive and life-threatening ones [9, 10].

PSMs were first identified in 1999 by the group of Seymour Klebanoff with the description of a “pro-inflammatory complex”. They were isolated by hot phenol extraction from *S. epidermidis* culture filtrate [11]. The peptides were named PSM α , PSM β and δ -toxin. Afterwards, the PSM composition of *S. aureus* was also analyzed more systematically, which include the shorter α -type (four PSM α 1-PSM α 4 peptides and δ -toxin) and the longer β -type (the PSM β 1 and PSM β 2 peptides). The PSMs are encoded at three different locations in the genome. Four PSM α 1-PSM α 4 peptides are encoded in the PSM α operon. PSM β 1 and PSM β 2 are encoded in the PSM β operon. δ -toxin is encoded within the coding sequence for RNAPIII [12, 13].

S. aureus are considered to be second to salmonella as important foodborne pathogens, which have been frequently reported as agents leading to outbreaks of diseases caused by enterotoxins in ready-to-eat food and food products. The pathogenic mechanisms of *S. aureus* have been examined extensively through different stages of infection. Various staphylococcal components contribute to virulence with an improved understanding of specific functions [14–16]. Proteomics studies have revealed that the production of virulence factors by different isolates of *S. aureus* is diverse and only a few of these seem to be invariantly produced [17]. PSMs are the most commonly identified staphylococcal virulence factors, especially in the community-associated (CA)-MRSA lineages. Recently, it was found that many *in vitro* PSM phenotypes were strongly inhibited by serum lipoproteins, and can exert their contribution to pathogenesis by intracellular killing and participate in neutrophil killing after phagocytosis [18]. In addition, PSMs at low concentration can lead to immune cell chemotaxis and inflammatory reaction. High concentration of PSMs may cause cytolysis, damaging neutrophils, immune cells, red blood cells and white blood cells [19, 20]. Thus, it is important to develop a rapid, specific and accurate method to detect PSMs in food products for the prevention of transmission.

At present, there are only a few methods for determination of PSMs, such as imaging mass spectrometry and liquid chromatography-ion trap or quadrupole mass spectrometry [11, 21]. The sample pre-treatment of the method using imaging mass spectrometry is quite cumbersome and time-consuming. The resolution of the method by liquid chromatography-ion trap or quadrupole mass spectrometry is too low to separate interfering substances with similar charge-to-mass ratios. Here, we describe a simple and effective method with higher sensitivity and selectivity for PSM analysis in *S. aureus* by high-performance liquid chromatography-quadrupole time-of-flight mass spectrometry system (HPLC-QTOF).

2. Materials

2.1. Reagents and chemicals

1. Tryptone soy broth (TSB).
2. *Staphylococcus aureus* strains.

3. 1-butanol.
4. Acetonitrile.
5. Formic acid.
6. Ultrapure water.
7. Eluent A: 0.1% formic acid in water, Eluent B: 0.1% formic acid in acetonitrile.

2.2. Instrument and equipment

1. Chromatograph: High-performance liquid chromatography-quadrupole time-of-flight mass spectrometry system (1260 HPLC system connected to a 6530 Accurate-Mass spectrometer Q-TOF/MS, Agilent, America).
2. Columns: Zorbax 300SB-C8 (2.1 μm , 4.6 $\times$ 150 mm, Agilent, America).
3. Mass Hunter software (Agilent, America).
4. Centrifuge (3-18K, Sigma, Germany).
5. Autoclave (DB-200, Systec, Germany).
6. Incubator shaker (ZWY-211B, ZhiCheng, China).
7. N-EVAP (Organomation, America).

3. Methods

3.1. Extraction of PSMs

Pre-cultures grown overnight from a liquid culture are used to inoculate bacterial cultures at 1:100 dilution.

3.1.1. Extraction of PSMs from TSB for qualitative analysis

1. The *Staphylococcus aureus* cells were removed by centrifugation for 10 min (5000 rpm, 4°C) after overnight growth (~16 h) in 100 mL of culture medium (TSB) at 37°C with shaking at 200 rpm.
2. Transfer supernatants to a 100-mL volumetric flask.
3. Add 1/3 (v/v) of 100% 1-butanol to the supernatants to make 25% (v/v) of 1-butanol.
4. Shake the solutions vigorously at 37°C for 2 h.
5. After brief centrifugation, collect upper (butanol) phases.
6. Dry the extract under nitrogen and redissolve the dried sample in ultrapure water.
7. Filter the solution through a 0.22- μm filter using a syringe before LC-MS analysis.

3.1.2. Extraction of PSMs from TSB and beverage for quantitative analysis

1. The *Staphylococcus aureus* cells were removed by centrifugation (5000 rpm, 10 min, 4°C) after overnight growth (~16 h) in 4 mL of culture medium (TSB or beverage) at 37°C with shaking at 200 rpm. The “beverage” means pure juice or juice beverage. Soda, water and coffee were not included.
2. Transfer 1.5 mL supernatants to 4-mL centrifuge tubes.
3. The supernatants were incubated at a 1:1 ratio with 1-butanol for 2 h (37°C, 200 rpm).
4. Transfer 1.0 mL supernatants to 2-mL centrifuge tubes.
5. Dry the extract under nitrogen and redissolve the dried sample in 0.5 mL ultrapure water.
6. Filter the solution through a 0.22-μm filter using a syringe before LC-MS analysis.

3.1.3. Extraction of PSMs from milk for quantitative analysis

1. The *Staphylococcus aureus* cells were precipitated by acetonitrile after overnight growth (~16 h) in 4 mL of culture medium (milk) at 37°C with shaking at 200 rpm.
2. Remove fat by frozen centrifugation technology (5000 rpm, 10 min, 0°C).
3. Extract water layer 1.5 mL to 4-mL centrifuge tubes.
4. The extract was incubated at a 1:1 ratio with 1-butanol for 2 h (37°C, 200 rpm).
5. Transfer 1.0 mL supernatants to 2-mL centrifuge tubes.
6. Dry the extract under nitrogen and redissolve the dried sample in 0.5 mL ultrapure water.
7. Filter the solution through a 0.22-μm filter using a syringe before LC-MS analysis.

3.1.4. Extraction of PSMs from pork for quantitative analysis

1. The *Staphylococcus aureus* cells were precipitated by acetonitrile after overnight growth (~16 h) in 4 mg of culture medium (pork) at 37°C with shaking at 200 rpm.
2. Add 1.5 mL 1-butanol and remove fat by frozen centrifugation technology (5000 rpm, 10 min, 0°C) after extraction of 2 h (37°C, 200 rpm).
3. Transfer 1.0 mL supernatants to 2-mL centrifuge tubes.
4. Dry the extract under nitrogen and redissolve the dried sample in 0.5 mL ultrapure water.
5. Filter the solution through a 0.22-μm filter using a syringe before LC-MS analysis.

3.2. Analysis of PSMs

3.2.1. Liquid chromatography methodology

1. Transfer the supernatants into glass sample vials. Cool down the sample tray to 10°C and put the sample vials in it.

2. An Agilent high-performance liquid chromatography-electrospray ionization-quadrupole time-of-flight mass spectrometry (HPLC-ESI-QTOF/MS) system was used to analyze the PSMs qualitatively and quantitatively.
3. The chromatographic separation was performed with a Zorbax 300SB-C8 column (2.1 μm , 4.6 $\times$ 150 mm, Agilent) in series with a flow rate of 0.5 mL/min.
4. The gradient program can be described as follows: 10% eluent B for 2.5 min; 50% eluent B for 2.5 min; a linear gradient from 50% to 90 % eluent B for 15 min.
5. Injections (10 μL) were made using an autosampler, and the column temperature was set at 30°C.

3.2.2. Qualitative analysis of PSMs from culture

1. Transfer the supernatants into glass sample vials. Cool down the sample tray to 10°C and put the sample vials in it.
2. The ESI experiments were carried out by Dual AJS ESI ion source in a high-resolution instrument mode with a mass range of 3200 m/z and a positive scan mode with gas temperature at 325°C, drying gas flow rate at 5 L/min, sheat gas temperature at 350°C, and sheat gas flow rate at 7.5 L/min. The nebulizing gas was produced by a nitrogen generator.
3. The capillary voltage was kept at 4000 V and the fragmentor voltage was set to 175 V. Signals acquired in MS mode were used to make a preliminary identification, and data obtained in targeted MS/MS mode were searched from the online Mascot database, which could determine the PSMs accurately.
4. The peak area based on extracted ion chromatogram (EIC) can be used to quantitate the PSMs sensitively and rapidly.

4. Results and discussion

Examples of preset m/z ratios and retention times of *S. aureus* PSMs for qualitative and quantitative analyses are shown in **Table 1**. Retention times may vary according to the LC system and MS detector. (d), (t) and (q) stand for doubly, triply and quadruply charged ions, respectively.

The chromatograms of PSMs extracted from the culture are shown in **Figure 1**. The result indicates that the new method has a high selectivity. Good linearity of the PSMs was achieved in the range 0.5~100 $\mu\text{g/L}$ ($R^2 > 0.99$). Compared with other analytical methods, the pre-treatment of the new method is simple and rapid, and the high resolution makes the method highly sensitive and selective. The method has been used successfully for the determination of PSMs extracted from different culture mediums, such as milk, beverage, vegetable and meat.

PSM	MS (m/z)	RT (min)	MS/MS (m/z)
PSM α 1	1144.6842(d)	14.353	330.1491, 302.1539
PSM α 2	1153.6964(d)	12.727	330.1475, 302.1538
PSM α 3	1318.2228(d)	11.705	289.0861, 436.1542
PSM α 4	1100.6982(d)	20.250	344.1674, 316.1711
δ -toxin	1002.8844(t)	15.010	474.1661, 587.2490
PSM β 1	1131.8610(q)	15.437	346.1059, 459.1892
PSM β 2	1121.8514(q)	11.353	318.1080, 702.3068

Table 1. m/z ratios and retention times of *S. aureus* PSMs.

Figure 1. Chromatograms of PSMs extracted from the culture.

Acknowledgements

This work was supported by Science & Technology Project of China General Administration of Quality Supervision, Inspection and Quarantine (2015QK055), Science & Technology Project of Quality Supervision Bureau of Jiangsu Province (KJ155426), Six talent peaks project in Jiangsu Province (SWYY-031) and Jiangsu Natural Science Foundation (BK20141085).

Author details

Xiaoxiao Wu and Chi Zhang*

* Address all correspondence to: zhangchi@njzj.gov.cn

National Center of Supervision Inspection on Processed Food and Food Additives Quality,
Nanjing Institute of Product Quality Inspection, Nanjing, China

References

- [1] Gonzalez DJ, Vuong L, Gonzalez IS, Keller N, McGrosso D, Hwang JH, Hung J, Zinkernagel A, Dixon JE, Dorrestein PC. Phenol soluble modulin (PSM) variants of community-associated methicillin-resistant *Staphylococcus aureus* (MRSA) captured using mass spectrometry-based molecular networking. *Molecular & Cellular Proteomics*. 2014; **13**:1262–1272. doi:10.1074/mcp.M113.031336.
- [2] Peschel A, Otto M. Phenol-soluble modulins and staphylococcal infection. *Nature Reviews Microbiology*. 2013; **11**:667–673. doi:10.1038/nrmicro3110.
- [3] Cheung GYC, Joo HS, Chatterjee SS, Otto M. Phenol-soluble modulins – critical determinants of staphylococcal virulence. *Fems Microbiology Reviews*. 2014; **38**: 698–719. doi:10.1111/1574-6976.12057.
- [4] Wang R, Braughton KR, Kretschmer D, Bach TH, Quech SY, Li M, Kennedy AD, Dorward DW, Klebanoff SJ, Peschel A, Deleo FR, Otto M. Identification of novel cytolytic peptides as key virulence determinants for community-associated MRSA. *Nature Medicine*. 2007; **13**:1510–1514. doi:10.1038/nm1656.
- [5] Cogen AL, Yamasaki K, Sanchez KM, Dorschner RA, Lai YP, Macleod DT, Torpey JW, Otto M, Nizet V, Kim JE, Gallo RL. Selective antimicrobial action is provided by phenol-soluble modulins derived from *Staphylococcus epidermidis*, a normal resident of the skin. *Journal of Investigative Dermatology*. 2009; **130**:192–200. doi:10.1038/jid.2009.243.
- [6] Joo HS, Cheung GY, Otto M. Antimicrobial activity of community-associated methicillin-resistant *Staphylococcus aureus* is caused by phenol-soluble modulin derivatives. *Journal of Biological Chemistry*. 2011; **286**:8933–8940. doi:10.1074/jbc.M111.221382. Epub 2011 Jan 28.
- [7] Wang R, Khan BA, Cheung GY, Bach TH, Jameson-Lee M, Kong KF, Queck SY, Otto M. *Staphylococcus epidermidis* surfactant peptides promote biofilm maturation and dissemination of biofilm-associated infection in mice. *Journal of Clinical Investigation*. 2011; **121**:238–248. doi:10.1172/JCI42520.
- [8] Periasamy S, Joo HS, Duong AC, Bach TL, Tan VY, Chatterjee SS, Cheung GYC, and Otto M. How *Staphylococcus aureus* biofilms develop their characteristic structure. *Proceedings of the National Academy of Sciences*. 2012; **109**:1281–1286. doi:10.1073/pnas.1115006109.

- [9] Lowy FD. *Staphylococcus aureus* infections. *New England Journal of Medicine*. 1998; **339**:520–532. doi:10.1056/NEJM199808203390806.
- [10] Peacock SJ, Silva ID, Lowy FD. What determines nasal carriage of *Staphylococcus aureus*? *Trends in Microbiology*. 2001; **9**:605–610. doi:10.1016/S0966-842X(01)02254-5.
- [11] Mehlin C, Headley CM, Klebanoff SJ. An inflammatory polypeptide complex from *Staphylococcus epidermidis*: isolation and characterization. *Journal of Experimental Medicine*. 1999; **189**:907–918. doi:10.1084/jem.189.6.907.
- [12] Queck SY, Khan BA, Wang R, Bach TH, Kretschmer D, Chen L, Peschel A, Deleo FR, Otto M. Mobile genetic element-encoded cytolysin connects virulence to methicillin resistance in MRSA. *PLoS Pathogens*. 2009; **5**:e1000533. doi:10.1371/journal.ppat.1000533.
- [13] Chatterjee SS, Chen L, Joo HS, Cheung GY, Kreiswirth BN, Otto M. Distribution and regulation of the mobile genetic element-encoded phenol-soluble modulins PSM-mec in methicillin-resistant *Staphylococcus aureus*. *PLoS One*. 2011; **6**:e28781. doi:10.1371/journal.pone.0028781.
- [14] Cheng AG, Kim HK, Burts ML, Krausz T, Schneewind O, Missiakas DM. Genetic requirements for *Staphylococcus aureus* abscess formation and persistence in host tissues. *Journal Official Publication of the Federation of American Societies for Experimental Biology*. 2009; **23**:3393–3404. doi:10.1096/fj.09-135467.
- [15] Dumont AL, Nygaard TK, Watkins RL, Smith A, Kozhaya L, Kreiswirth BN, Shopsin B, Unutmaz D, Voyich JM, Torres VJ. Characterization of a new cytotoxin that contributes to *Staphylococcus aureus* pathogenesis. *Molecular Microbiology*. 2011; **79**:814–825. doi:10.1111/j.1365-2958.2010.07490.x.
- [16] Cheung GYC, Duong AC, Otto M. Direct and synergistic hemolysis caused by *Staphylococcus*, phenol-soluble modulins: implications for diagnosis and pathogenesis. *Microbes & Infection*. 2012; **14**:380–386. doi:10.1016/j.micinf.2011.11.013.
- [17] Breshears LM, Gillman AN, Stach CS, Schlievert PM, Peterson ML. Local Epidermal growth factor receptor signaling mediates the systemic pathogenic effects of *Staphylococcus aureus* toxic shock syndrome. *PLoS One*. 2016; **11**:e0158969. doi:10.1371/journal.pone.0158969.
- [18] Surewaard BG, Nijland R, Spaan AN, Kruijtz JA, de Haas CJ, van Strijp JA. Inactivation of staphylococcal phenol soluble modulins by serum lipoprotein particles. *PLoS Pathogens*. 2012; **8**:e1002606. doi:10.1371/journal.ppat.1002606.
- [19] Otto M. Phenol-soluble modulins. *International Journal of Medical Microbiology*. 2014; **304**:164–169. doi:10.1016/j.ijmm.2013.11.019.
- [20] Kretschmer D, Gleske AK, Rautenberg M, Wan R, Koberle M, Bohn E, Schoneberg T, Rabiet MJ, Boulay F, Klebanoff SJ, van Kessel KA, van-Strijp JA, Otto M, Peschel A.

Human formyl peptide receptor 2 senses highly pathogenic *Staphylococcus aureus*. *Cell Host Microbe*. 2010; **7**:463–473. doi:10.1016/j.chom.2010.05.012.

- [21] Gonzalez DJ, Okumura CY, Hollands A, Kersten R, Akong-Moore K, Pence MA, Malone C, Derieux J, Moore BS, Horswill AR, Dixon JE, Dorrestein PC, Nizet V. Novel phenol-soluble modulin derivatives in community-associated methicillin-resistant *Staphylococcus aureus* identified through imaging mass spectrometry. *Journal of Biological Chemistry*. 2012; **287**:13889–13898. doi:10.1074/jbc.M112.349860.