Food Control 72 (2017) 153–163

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Affinity and selectivity of anchovy antibacterial peptide for *Staphylococcus aureus* cell membrane lipid and its application in whole milk

Chuanfen Pu, Wenting Tang*

School of Food Science and Engineering, Qingdao Agricultural University, Qingdao 266109, China

ARTICLE INFO

Article history:
Received 14 April 2016
Received in revised form
10 July 2016
Accepted 2 August 2016
Available online 4 August 2016

Keywords:
Antimicrobial peptide
Cell membrane
Selectivity
Staphylococcus aureus

ABSTRACT

An anchovy antibacterial peptide ACWWP1 (GLSRLFTALK) has been previously shown to kill bacterial cells via membrane damage. The affinity and selectivity of ACWWP1 for cell membrane lipids and their effects on the activity were investigated. Thermodynamic analysis indicated that hydrophobic and electrostatic effects contributed to the partitioning of ACWWP1 in the membrane lipids. Confocal Raman microspectroscopy, fluorescence anisotropy and calcein leakage analysis suggested that the peptide induced the greatest change in the order parameters of the *Staphylococcus aureus* cell membrane, which was related to the relaxation of the lateral stacking and an increase in the fluidity of the phospholipid bilayer. The positively charged ACWWP1 could change the surface charge state of the membrane. Besides, ACWWP1 exhibited efficient antimicrobial activity for *S. aureus* in whole milk. The results suggest that ACWWP1 exhibits favorable bacterial cell membrane lipid selectivity and can be employed as an efficient method for the treatment of food poisoning caused by certain bacteria.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

Antimicrobial peptides (AMPs) are evolutionarily recognized as promising candidates to overcome the urgent problems of limited safety of chemical food preservatives and antibiotic resistance of multidrug-resistant bacteria. AMPs could be used as new food preservatives due to their potential advantages. They exert an antibacterial effect through interaction with the bacterial cell membrane or intracellular macromolecules, or by interfering with bacterial metabolism. The diversity of the amino acid sequence and structure of AMPs determined the differences in the antibacterial activity and antibacterial spectrum of various AMPs (Reddy, Yedery, & Aranha, 2004). No unified theory exists to clarify the antibacterial mechanism of AMPs. The view that most AMPs first interact with the bacterial cell membrane when exerting their antibacterial activities has been commonly recognized (Hancock & Rozek, 2002). Disintegration and permeation are the two main forms of cell membrane action for AMPs, including carpet, barrel-stave, toroidal pore, sinking-raft, and other models (Pálffy et al., 2009; Xiao, Zhang, & Ding, 2012; Yeaman & Yount, 2003). It has been proposed that cationic residues and hydrophobic amino acids contribute to the antibacterial activities of AMPs (Torrent, Nogués, & Boix, 2009). The electrostatic interaction attracts the cationic AMPs to the negatively charged bacterial surface, whereas hydrophobic interaction creates the antibacterial peptide partition in the membrane lipid bilayer (Giuliani, Pirri, & Nicoletto, 2007; Yeaman & Yount, 2003).

The interactions between the AMPs and the cell membrane are no doubt related to the peptide structure characteristics, including the amino acid sequence, the molecular size, the charge state, and the hydrophobic properties. They are also closely linked with the composition and arrangement of phospholipids in the bacterial cell membrane. The phospholipid bilayer is the basic skeleton of the bacterial cell membrane. In the membrane action process, AMPs generally prefer to interact with specific phospholipids, but not the protein receptors, in the cell membrane (Lohner & Prenner, 1999). Previous studies of the interaction between AMPs and liposome, a kind of cell membrane model, demonstrated that some AMPs might display membrane selectivity. These peptides may have stronger interactions with negatively charged liposome than with electrically neutral liposome, which would indicate that the charge state of the phospholipid in the cell membrane contributes to the membrane selectivity action (Fernandez, Lee, Sani, Aguilar, &

* Corresponding author.
E-mail address: twtfly@126.com (W. Tang).

http://dx.doi.org/10.1016/j.foodcont.2016.08.009
0956-7135/© 2016 Elsevier Ltd. All rights reserved.

Separovic, 2013; Marcotte et al., 2003). Moreover, the hydrophobic interaction is affected by the hydrophobic tail of the phospholipids and the hydrophobic residues of the peptide. Thus, for a particular peptide, the chemical properties of the phospholipid headgroup and the side chain, such as the electric charge and fatty acid composition, play important roles in the selective action on the eukaryotic cells and prokaryotic cells, including different species of bacteria cells (Ishitsuka, Pham, Waring, Lehrer, & Lee, 2006). Therefore, the differences in the membrane components of the target cells may cause varying degrees of inhibition for a certain peptide on the different strains.

Food poisoning caused by *Staphylococcus aureus* is a concerning food safety issue. Based on the interaction between AMPs and the bacterial cell membrane, an antibacterial peptide ACWWP1 (GLSRLFTALK) has been purified from the protein hydrolysate of anchovy (*Engraulis japonicus*) cooking wastewater and the membrane damage mechanism of ACWWP1 has been proved (Tang, Zhang, Wang, Qian, & Qi, 2015). It has been found that ACWWP1 exhibited potent antimicrobial activity against *S. aureus* with a minimum inhibitory concentration of 16 μg/mL. However, whether ACWWP1 shows the membrane lipids selectivity and this in turn leads to different activity against bacteria requires further verification. Further, the activity determined in favorable culture media may not be applicable to the real food matrix.

Regarding these aspects, we constructed three cell membrane models from mixed commercial phospholipid, *Escherichia coli* lipid, and *S. aureus* lipid to investigate the membrane lipid selectivity of ACWWP1. The interactions between the antibacterial peptide and the three membranes were characterized. The structural changes in the lipid bilayers in the presence of the peptide were revealed. The anti-*S. aureus* activity of ACWWP1 in whole milk was also evaluated.

2. Materials and methods

2.1. Materials

Egg-yolk phosphatidylcholine (EYPC) and 1, 2-Dimyristoyl-*sn*-glycero-phosphatidylglycerol (DMPG) were obtained from A. V. T. Pharmaceutical (Shanghai, China). *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 were provided by School of Food Science and Engineering, Qingdao Agricultural University. Luria-Bertani (LB) medium was purchased from Hope Biotechnology Co., Ltd. (Qingdao, China). Macroporous spherical silica (particle diameter 5 μm, pore diameter 120 Å) was purchased by Sepax Technologies (Suzhou, China). 1,6-diphenyl-1,3,5-hexatriene (DPH, 98% purity) was purchased from Molecular Probes. Calcein was purchased from Sangon Bioeng (Shanghai, China). Fluorescein isothiocyanate (FITC) were purchased from Aldrich Chemical Company (St. Louis, MO, US). All other chemicals were of analytical grade and used without further purification.

2.2. Peptide synthesis

Anchovy antibacterial peptide ACWWP1 (GLSRLFTALK) was synthesized using standard 9-fluorenylmethoxycarbonyl (FMOC) solid-phase method on a model 432A peptide synthesizer (Applied Biosystems, Foster City, CA), and purified to >98% purity by RP-HPLC on a C18 column. The exact molecular mass and sequence of the purified peptide were confirmed by mass spectroscopy analysis.

2.3. Total lipid extraction of bacterial cell membranes

Total lipid extraction of membranes of *E. coli* and *S. aureus* was conducted according to the previous method (Tang, Zhang, Wang, & Qian, 2014). Both strains were grown in LB broth at 37 °C. At mid-log phase, about 30 g of wet cells were collected and re-suspended in 180 mL of Tris buffer (25 mM, pH 7.5). And then, 600 mL of chloroform–methanol mixture (1:2, v/v) was added to the suspension. The whole sample was then sonicated for 2 min. After sonication, further 200 mL of chloroform and 200 mL of distilled water were added, and the mixture was again sonicated for 2 min. The chloroform phase was separated, and then the solvent was removed by a rotary evaporator under vacuum.

2.4. Determination of partition of ACWWP1 in liposome

2.4.1. Preparation of immobilized liposome stationary phase (ILSP)

Macroporous spherical silica was activated according to the previously reported method (Tang et al., 2014). The lipid was dissolved in chloroform/methanol (15:1, v/v) and activated silica was added to the solution, followed by shaking for 60 min at 4 °C. The solvent was removed by rotary evaporation, and then the dried material obtained was treated with nitrogen and kept under vacuum overnight to remove the remaining solvent. The lipid film coated porous silica was swollen for 6 h to form liposome. Finally, the silica was washed with Tris buffer until there was no phosphorus detected in the supernatant in order to remove non-immobilized liposome. Phosphorus was determined according to the method of Bartlett (Bartlett, 1959).

2.4.2. Chromatographic conditions

The obtained ILSP was packed into a stainless steel column with the dimension of 150 mm length × 4.6 mm i.d. via the slurry packing method. The mobile phase used in the experiment was sodium phosphate buffer (PBS, pH 7.2, 10 mM) containing 50 mM NaCl. The flow-rate of the mobile phase was 0.5 mL/min and the UV detection wavelength was monitored at 215 nm. The column was equilibrated with the mobile phase before sample injection.

2.4.3. Determination of capacity factor of ACWWP1 in immobilized liposome chromatography (ILC)

The interaction between the ACWWP1 and the liposomes was expressed as the capacity factor (K), which was calculated by the following equation (Beigi et al., 1998):

$$K = (V_R - V_0)/A,$$

where V_R is the retention volume of the peptide on ILC column; V_0 is the void volume of the reference molecule ($NaNO_3$), which does not interact with the liposomes or the silica; and A is the amount of immobilized phospholipid amount in the column, which was determined by phosphorus analysis (Bartlett, 1959). The effects of temperature on the partitioning and the thermodynamic parameters of ACWWP1 were investigated under different temperatures. The thermodynamic parameters including enthalpy change (ΔH), entropy change (ΔS), and free energy change (ΔG) can be evaluated using the following equations:

$$\ln K = -\Delta H/RT + \Delta S/R$$

$$\Delta G = \Delta H - T x \Delta S$$

where R is the gas constant, T is the absolute temperature.

2.5. Confocal Raman microscope analysis

The cell membrane model, liposome, was prepared by the thin-film evaporation method (Varona, Martín, & Cocero, 2011). Briefly,

Download English Version:

https://daneshyari.com/en/article/4558959

Download Persian Version:

https://daneshyari.com/article/4558959

Daneshyari.com