Antibacterial characteristics of glycinin basic polypeptide against *Staphylococcus aureus*

Jie Yang, Gui-Jin Sun, Ying-Qiu Li*, Kai-Yu Cui, and Hai Zhen Mo¹

School of Food Science and Engineering, Qilu University of Technology, No. 3501 University Road of Changqing District, 250353, Jinan, Shandong, China

¹School of Food Science, Henan Institute of Science and Technology, Xinxiang, 453003, China

Received March 2, 2016
Revised August 1, 2016
Accepted August 4, 2016
Published online October 31, 2016

*Corresponding Author
Tel: +86-531-89631195
Fax: +86-531-89631195
E-mail: lyq@qlu.edu.cn; sdililyq@126.com

pISSN 1226-7708
eISSN 2092-6456

© KoSFoST and Springer 2016

Abstract This paper aims to study the antibacterial action of glycinin basic polypeptide (GBP) on *Staphylococcus aureus* (*S. aureus*). Herein, the minimum inhibitory concentration (MIC) of GBP against *S. aureus* was 0.2 mg/mL. Atomic force microscopy (AFM) imaging showed that GBP seriously damaged the morphology of the *S. aureus* cells. GBP (0.8 mg/mL) enhanced the relative release of β -galactosidase to 25.48% when compared to the control. The activity of the respiratory-chain dehydrogenase of *S. aureus* decreased with increasing GBP concentration. GBP could cause a leakage of intracellular substances. Additionally, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) demonstrated that *S. aureus* bacterial proteins decreased in response to the time period of treating the bacterial cells with GBP. These results indicate that GBP could remarkably inhibit *S. aureus* and is, therefore, a potential food preservative.

Keywords: glycinin basic polypeptide, novel antibacterial agent, antibacterial action, *Staphylococcus aureus*

Introduction

Food preservatives can prevent the growth of pathogenic and spoilage microorganisms such as *Staphylococcus aureus* (*S. aureus*), which may threaten human health (1). Chemical synthetic preservatives have aroused wide public concern because of their many negative effects on human health if used continuously (2). Therefore, the exploitation and utilization of novel natural food preservatives have received increasing interest from the food industry and researchers (3).

Several antimicrobial peptides (AMPs) play an important role in food preservation as novel antimicrobial agents (4). These AMPs, which are generally isolated from different types of organisms, including bacteria, plants, and animals, exhibit significant antibacterial activities (5). The AMP ST4V, produced by *Enterococcus mundtii* ST4V, is an active antibacterial agent against Gram-positive and Gram-negative bacteria such as *Enterococcus faecalis*, *Streptococcus* spp., *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, and *S. aureus* (6). Recombinant antifungal defensin (rAFP1), derived from Brassica juncea, inhibits the growth of wild-type *Candida albicans* cells at a concentration range of 1–10 μ g/mL (7). Park *et al.* (5) reported that the minimum inhibition concentrations (MICs) of misgurin (isolated from *Misgurnus anguillicaudatus*)

against *S. aureus*, *Salmonella typhimurium*, *Pseudomonas putida*, *Bacillus subtilis*, and *Escherichia coli* (*E. coli*) are all 8 μ g/mL.

Some studies (8,9) have reported that known AMPs might provide antimicrobial action by interacting with the membrane of a bacterial cell, thereby damaging it. AMP aurein 1.2 can bind to the surface of a cell membrane and disrupt it at a threshold concentration of 10 μ g/mL (8). However, Pep²⁷ (27-amino acid peptide) penetrates the bacterial membrane of *Staphylococcus epidermidis* KCTC 1917 and *Pseudomonas aeruginosa* ATCC 27853 via an energy-independent pathway and does not damage the membrane (10).

Glycinin basic polypeptide (GBP), a basic subunit of soybean glycinin, is a cationic peptide with a molecular weight of approximate 20 kDa and is linked to the acid subunit of soybean glycinin by a single disulfide bond (11,12). GBP is a colorless, odorless white powder and exhibits low solubility in water and hydrophobicity. GBP has cationic characteristics, i.e., it can attract the membrane of a bacterial cell and damage it (11–15). Moreover, animal toxicity was not observed in the glycinin basic subunit (i.e. GBP) (16).

Osman *et al.* (16) reported that the glycinin basic subunit (6.25–100 μ g/mL) inhibits vigorously resistant-vancomycin intermediate *S. aureus* (VISA). Moreover, the antimicrobial activity of the glycinin basic subunit against VISA P59 is stable over a wide pH range and at different incubation temperatures. It can inhibit almost 99% of the

viable growth of VISA P59 in minced beef meat stored at 37°C for 14 days. GBP exhibits antibacterial activity against *Listeria monocytogenes* Scott A, *Bacillus subtilis*, and *Salmonella enteritidis* with 50 µg/mL of minimum inhibitory concentration (MIC). Moreover, GBP significantly inhibits the propagation of *Listeria monocytogenes*, *Salmonella enteritidis*, and *Bacillus subtilis* inoculated in pasteurized milk during 16–20 days of storage at 4°C (12). Thus, GBP may be used as substitute or supplement for synthetic preservatives in food because of its bacteriostasis.

However, only a few studies have reported the antibacterial mechanisms of GBP, especially against *S. aureus*, which is the common pathogenic and spoilage bacteria in food. Thus, the aim of this study is to study the antibacterial actions of GBP on *S. aureus*. The antibacterial characteristics of GBP were evaluated via the plate counting method and atomic force microscopy (AFM) analysis. The antibacterial action of GBP on *S. aureus* was assessed on the basis of the changes in the leakage of β-galactosidase, activity of the respiratory-chain dehydrogenase, and constituents of the *S. aureus* cells.

Materials and Methods

Bacterial strain and inoculum preparation *S. aureus* ATCC 6538 was obtained from the Culture Collection, Qilu University of Technology. Freeze-dried bacteria were activated according to the ATCC guidelines. The strain was maintained on the slants of the beef extract-peptone medium (BEP, 0.3% beef extract, 1% peptone, 0.5% NaCl, 2% agar, and 96.2% sterile distilled water). One single colony of culture was inoculated into a flask containing 100 mL of sterile BEP broth. The inoculated broth was then incubated at 37°C in a water bath with shaking (130 rpm) for 10 h in order to achieve the stationary phase. The final cell concentration was approximately 10⁸–10⁹ colony-forming units per milliliter (CFU/mL), which was used for subsequent studies.

Preparation and purification of GBP Defatted soybean flakes purchased from Scents Holding Co., Ltd., (Jinan, China), were ground to pass through a 1-mm sieve, and the obtained powder was used for the isolation of glycinin according to the method reported by Nagano *et al.* (17). Glycinin was dissolved in 30 mM Tris buffer (pH=8.0, containing 15 mM β-mercaptoethanol) to a final concentration of 20 mg/mL. The protein solution was heated to 90°C for 30 min and then centrifuged (11,000×g) at 4°C for 15 min. The precipitate (crude GBP) was washed thrice with a small amount of the 30 mM Tris buffer (pH=8.0) dispersed in distilled water, and freeze-dried. Crude GBP was dissolved in 10 mM phosphate buffer (pH=7.2) to a final concentration of 1 mg/mL and then eluted with the same phosphate buffer (pH=7.2) by Sephadex G-150 chromatography at 2 mL/min. The eluent containing purified GBP was collected and freeze-dried into a powder for the experiments.

Antibacterial activity assay The antibacterial effect of GBP on *S. aureus* cells was determined using a conventional plate counting method. *S. aureus* cells from the mid-logarithmic phase were harvested and diluted with 10 mM phosphate-buffered saline (PBS, pH=7.4; 137 mM NaCl, 2.7 mM KCl, 4.3 mM Na₂HPO₄, and 1.4 mM KH₂PO₄) to a final concentration of 10⁶ CFU/mL. GBP was added to the aliquot mixtures to final concentrations of 0 (the control), 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1 mg/mL. Then, the mixtures were incubated at 37°C with shaking for 90 min. Subsequently, an appropriate dilution series of the samples with a saline solution was plated on nutrient agar plates and incubated at 37°C for 24 h. The colony-forming units (CFU) were determined via standard plate-counting procedures.

Atomic force microscopy (AFM) analysis Aliquots (4 mL) of the *S. aureus* culture (approximately 10⁸ CFU/mL) treated with GBP at 0 (the control) and 0.4 mg/mL were incubated at 37°C (150 rpm) for 4 and 8 h, respectively, and then centrifuged at 7,000×g for 10 min. The pellets were suspended in fresh BEP broth to the original volume of the aliquots (4 mL). The suspension was centrifuged again at 7,000×g for 10 min. Bacterial cells were fixed with 4 mL of the 20 mg/mL glutaraldehyde at 4°C for 12 h. Then, bacterial cells were harvested via centrifugation at 7,000×g for 10 min and resuspended in 4 mL of double-distilled water. Two drops of the cell suspensions were placed on a clean glass slide (1×1 cm), and the slides were then dried at 37°C for 12 h. The samples were detected via AFM (Mutimode 8 Nanoscope V system, Bruker, Billerica, MA, USA).

Measurement of β-galactosidase of *S. aureus* cells The measurement of the amount of cytoplasmic β-galactosidase released from *S. aureus* into the culture medium was performed as described in a study (18). Bacterial cultures (approximately 10⁸ CFU/mL) were infused with a biochemical incubator (SHP-150; Shanghai Jing Hong Laboratory Instrument Co., Ltd., Shanghai, China) containing GBP at different final concentrations (0, 0.2, 0.4, 0.6, and 0.8 mg/mL) at 37°C for 1 h. Then, the samples were centrifuged at 4,500×g for 15 min. An aliquot (4.5 mL) of the collected supernatant was incubated with 0.5 mL of o-nitrophenyl-β-D-galactoside (ONPG, 1 mg/mL) at 37°C for 3.5 h. The hydrolysis of ONPG to o-nitrophenol after 3.5 h was monitored on the basis of the optical density (OD) of the suspension at 415 nm using a spectrophotometer (TU-1901; Beijing Purkinje General Instrument Co., Ltd., Beijing, China), and the resulting OD₄₁₅ values were marked as OD_α. Bacterial suspension (approximately 10⁸ CFU/mL) without GBP treatment was sonicated (600 W) at 25°C for 15 min using an ultrasonic cleaner (BK-600B; Bakr Ultrasonic Technology Co., Ltd., Jinan, China) and then prepared according to the procedure described above for spectrophotometry; its OD₄₁₅ value was marked as OD_β. The relative release of β-galactosidase was defined as

$$\text{Relative release (\%)} = \frac{\text{OD}_{\alpha}}{\text{OD}_{\beta}} \times 100$$

Measurement of the respiratory-chain dehydrogenase of *S. aureus* cells 2,3,5-triphenyltetrazolium chloride (TTC), a small molecule, can penetrate into bacterial cells and is sensitive to degradation by respiratory-chain dehydrogenase in bacterial cells. After a series of reduction reactions, TTC is transformed into triphenylformazone (TF), which has a maximum absorption peak at approximately 490 nm (19-21).

The respiratory-chain dehydrogenase activity was measured via the method of Klapwuk *et al.* (22) with some modifications. An aliquot (1 mL) of the culture solution of *S. aureus* (approximately 10^8 CFU/mL) was mixed with 50 mM Tris-HCl buffer (pH=8.6), 0.1 M glucose solution, and TTC (1 mg/mL), in that order. GBP was added to the aliquot mixtures to final concentrations of 0, 0.2, 0.4, 0.6, 0.8, and 1 mg/mL. The samples were inoculated at 37°C for 6 h. Then, two drops of 98% concentrated sulfuric acid was added to the samples to terminate the reactions. The suspension was mixed with 5 mL of n-butyl alcohol and shaken. After settling for 10 min, a microscope was used to observe and take photos of the suspension. The supernatant organic phase was moved and centrifuged at $3,000\times g$ for 6 min. The activity of the respiratory-chain dehydrogenase was then calculated according to the maximum absorbance of TF in the supernatant at 490 nm determined using a TU-1901 spectrophotometer.

Measurement of the leakage level of constituents in *S. aureus* cells GBP was added to the cell suspensions of *S. aureus* (approximately 10^8 CFU/mL) in 10 mM PBS (pH=7.4) to final concentrations of 0, 0.2, 0.4, 0.6, and 0.8 mg/mL. The cell suspensions were incubated at 37°C (150 rpm) for 2 h and then filtered through a 0.22 μ m microporous membrane to remove the bacteria cells. The amount of leaked cell constituents into the supernatant was determined by measuring its absorbance (OD) at 260 nm using a TU-1901 spectrophotometer at room temperature (25°C).

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of the proteins of *S. aureus* cells SDS-PAGE of bacterial proteins was performed according to the method reported by Li *et al.* (2) with some modifications. GBP was added to 18 mL suspensions of *S. aureus* (approximately 10^8 CFU/mL) to final concentrations of 0 (control) and 0.4 mg/mL. The bacterial suspensions of the control group were immediately centrifuged at $4,500\times g$ for 15 min. After shaking at 37°C for 30, 60, 90, 120, 150, 180, 210, and 240 min, the GBP-treated bacterial suspensions were also centrifuged at $4,500\times g$ for 15 min. The obtained pellets were washed twice with 10 mM PBS (pH=7.4) and resuspended in 400 μ L of 10 mM PBS (pH=7.4) and 300 μ L of lysozyme (10 mg/mL in 10 mM Tris-HCl, pH=8.0). Then, the mixture was incubated at 37°C for 50 min in a waterbath. Aliquots (100 μ L) of the cell suspensions were combined with 50 μ L of the sample buffer (pH=6.8; 1 M Tris-HCl, 50% glycerol, 10% SDS, 10% β -mercaptoethanol, and 0.1% bromophenol blue). This solution was then heated to 100°C for 10 min, cooled to room temperature (25°C),

and centrifuged at $7,000\times g$ for 10 min. Subsequently, the supernatant was loaded onto the 3% stacking and 12% resolving gels. The proteins were run through the stacking and resolving gels at 10 and 20 mA, respectively. The gel was dyed with Coomassie Brilliant Blue R250 and decolorized using a decoloring agent (glacial acetic acid, methanol, and distilled water). After four days, the protein bands were visualized on the gels.

Statistical analysis Every experiment was performed in triplicate, and the average values with standard error were reported. The Statistical Product and Service Solutions (SPSS) 17.0 software was used for all statistical analyses (SPSS Inc., Chicago, IL, USA). The data were analyzed via analysis of variance (ANOVA) and Duncan's post hoc analysis. Additionally, graphs were produced using Microsoft Excel 2007, and the regression analysis was used to determine the significant differences with 95% confidence ($p<0.05$).

Results and Discussion

Antibacterial effect of GBP on *S. aureus* As shown in Fig. 1, the total number of survival *S. aureus* cells was reduced by over 4 log CFU/mL after treatment with 1 mg/mL GBP for 90 min. This shows that the antibacterial activity of GBP was significantly greater than that of the control ($p<0.05$). The survival rate of *S. aureus* was less than 50% after treatment with 0.5 mg/mL of GBP for 90 min. The number of survival *S. aureus* cells was decreased with increasing GBP concentration. These data show that GBP has significant antibacterial effects against *S. aureus*. The MIC of GBP for *S. aureus* was 0.2 mg/mL.

Morphological changes in the *S. aureus* cells treated with GBP The morphological changes in the *S. aureus* cells treated with 0.4 mg/mL GBP for 4 and 8 h were examined via AFM (Fig. 2). As shown in Fig. 2A, the morphology of the untreated *S. aureus* cells was distinctly different from that of the *S. aureus* cells treated with GBP. In contrast to the *S. aureus* cells treated with GBP, the untreated ones had a smooth surface and an intact shape. There were deeper indentations and more wrinkles on the surfaces of the cells treated with 0.4 mg/mL GBP for 4 h in comparison to the untreated ones (Fig. 2B). Increasing the treatment time to 8 h resulted in more severe morphological changes compared with the treatment time of 4 h; numerous irregular grains and furrows were observed on the surfaces of the cells treated with GBP for 8 h (Fig. 2C).

This phenomenon was similar to that observed by Neethirajan and DiCicco (23), who reported that fosfomycin damages cell integrity and causes cell-surface degradation in *Staphylococcus pseudintermedius*. In an AFM observation conducted in a study, it was observed that all *E. coli* cells became thin and shriveled after treatment with copper-bearing austenitic antibacterial stainless steel (24). Additionally, Li *et al.* (25) on the basis of AFM results suggested

Fig. 1. Effect of different concentrations of GBP on log CFU/mL of *S. aureus* evaluated using the conventional plate counting method. The tested GBP concentrations were 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1 mg/mL.

that sushi peptides could disintegrate cell membranes of *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 and cause cytoplasm leakage.

Effect of GBP on the leakage of β -galactosidase from *S. aureus* cells

Figure 3 shows the effect of GBP on the leakage of β -galactosidase from *S. aureus* cells. As depicted in Fig. 3, the relative percentages of

β -galactosidase released from the *S. aureus* cells treated with 0, 0.2, 0.4, 0.6, and 0.8 mg/mL GBP were 0.42, 10.4, 17.8, 21.5, and 25.9%, respectively, with more than 60 times the relative release of β -galactosidase from the cells treated with 0.8 mg/mL than that from the untreated cells.

This indicated that GBP causes a considerable release of β -galactosidase from bacterial cells. The possible reason for the leakage of β -galactosidase from *S. aureus* cells is the disruption of the bacterial cell structure by GBP. This result is in agreement with the findings of Jang *et al.* (26), who, on the basis of the results of a β -galactosidase assay, reported that melittin exhibits strong cytoplasmic-membrane penetrability in bacteria. Similarly, Xing *et al.* (27) found that oleoyl-chitosan nanoparticles cause the release of cytoplasmic β -galactosidase from *E. coli* by increasing the permeability of the inner membrane.

Effect of GBP on the activity of the respiratory-chain dehydrogenase in *S. aureus* cells

The change in color of the samples treated with GBP is shown in Fig. 4A. The coloration of the samples decreased with increasing concentrations of GBP (from 0.2 to 1 mg/mL). The control had the reddest color of all the samples, indicating the maximum respiratory-chain dehydrogenase activity. Conversely, the sample treated with 1 mg/mL of GBP showed nearly no red

Fig. 2. Atomic force microscopy (AFM) images of the untreated and treated *S. aureus* cells under different conditions (left panels show topography images based on height data, the middle panels show deflection images based on peak force error data, and the right panels are three-dimensional (3D) images). (A) Control (untreated *S. aureus* cells); (B) *S. aureus* cells treated with 0.4-mg/mL GBP for 4 h; (C) *S. aureus* cells treated with 0.4-mg/mL GBP for 8 h.

Fig. 3. Relative release of β -galactosidase (measured by absorbance at 420 nm) from the *S. aureus* cells treated with 0, 0.2, 0.4, 0.6, and 0.8 mg/mL of GBP.

Fig. 4. Effect of GBP on the respiratory-chain dehydrogenase in *S. aureus* cells. (A) Photos of the coloration of samples with different concentrations of GBP. Samples 1, 2, 3, 4, 5, and 6 were the *S. aureus* cells treated with 0, 0.2, 0.4, 0.6, 0.8, and 1-mg/mL GBP, respectively; (B) OD₄₉₀ values of the samples treated with 0, 0.2, 0.4, 0.6, 0.8, and 1-mg/mL GBP.

coloration, indicating no respiratory-chain dehydrogenase activity. The differences in the OD₄₉₀ values of the treated samples compared with those of the control samples were in agreement with the differences in the coloration of the treated samples compared with those of the control samples (Fig. 4B). Figure 4B shows that the respiratory-chain dehydrogenase activity in the cells treated with GBP (even at a low concentration of 0.2 mg/mL) was significantly lower than that in the untreated cells. Thus, the respiratory-chain dehydrogenase activity was markedly inhibited by GBP, where the inhibition extent was inversely proportional to GBP concentration. The reduction in the respiratory-chain dehydrogenase activity caused by GBP might inhibit normal respiration of the cells, hinder the

Fig. 5. Effects of GBP on the leakage of intracellular substances (measured by absorbance at 260 nm) from *S. aureus* cells.

growth of bacteria, and even kill bacteria.

The results of this study are in agreement with the finding of Du *et al.* (28), who reported that hypocrellin A might destroy the respiratory-chain dehydrogenase proteins in *S. aureus*, thereby resulting in respiration inhibition. The same phenomenon occurred in *S. aureus* cells exposed to silver nanoparticles (29). Moreover, other researchers found that antibacterial agents inhibit the bacterial respiratory chain via inhibition of NADH oxidase, a constituent of the respiratory chains in bacterial membranes (30,31).

Effect of GBP on the leakage of intracellular substances The effect of GBP on the leakage of the intracellular substances from *S. aureus* is shown in Fig. 5. The OD₂₆₀ values of the *S. aureus* cells treated with 0.2, 0.4, 0.6, and 0.8 mg/mL GBP significantly increased to 0.147, 0.175, 0.203, and 0.231, respectively (Fig. 5). The leakage of intracellular substances was highest (OD₂₆₀=0.231) when the *S. aureus* cells were exposed to 0.8 mg/mL GBP for 2 h. In comparison to the control samples, a significant increase in the intracellular substances leakage from GBP-treated *S. aureus* cells was observed.

The significant leakage of intracellular substances from *S. aureus* indicated that GBP permeates and disrupts the cell membrane structure. This result was in agreement with the finding of Moosavy *et al.* (32), who reported a significant increase in the release of the 260 nm absorbing material of *S. aureus* treated with essential oil and nisin. Similarly, Tang *et al.* (33) reported that Opep2 disrupts the plasma membrane and causes nucleotide leakage, as indicated by the observed OD value at 260 nm. Additionally, Tang *et al.* (34) reported that MDpep9 causes nucleotide leakage from *E. coli* cells.

Effect of GBP on the bacterial proteins of *S. aureus* cells SDS-PAGE of the bacterial proteins from the *S. aureus* cells treated with 0.4 mg/mL GBP is shown in Fig. 6. Lanes M, 1, 2, 3, 4, 5, 6, 7, 8, and 9 were the marker, the control, and samples treated with 0.4 mg/mL GBP for 30, 60, 90, 120, 150, 180, 210, and 240 min, respectively. As seen in Fig. 6, the protein profiles of the bacteria treated with GBP differed from those of the control. In general, the content and types of bacterial proteins reduced as the treatment time increased. In comparison to the control, the bacterial protein bands with a

Fig. 6. SDS-PAGE of the bacterial proteins of the *S. aureus* cells treated with 0.4-mg/mL GBP for different time periods. Lane 1 is the control. Lanes 2–9 are the *S. aureus* cells treated with GBP for 30, 60, 90, 120, 150, 180, 210, and 240 min, respectively.

molecular weight lower than 40 kDa were significantly reduced at 30 and 60 min of GBP treatment (Fig. 6, lanes 2 and 3). However, bacterial proteins with a molecular weight higher than 40 kDa showed almost no change at 30 and 60 min of treatment. After 90 min of treatment, the amount and types of the bacterial protein bands with a molecular weight more than 40 kDa in the treated samples (Fig. 6, lanes 4–9) were greatly reduced in comparison to the control.

There are two possible explanations for the disappearance of the bacterial protein bands. First, GBP may have destroyed the *S. aureus* cell membrane, causing leakage of proteins from the cell. Additionally, destruction of the cell membrane may have hindered the transport of nutrients, causing the bacteria to utilize its cellular proteins to maintain its physiology and biochemical metabolic processes. The decrease in the number of protein bands with a molecular weight of less than 40 kDa occurred prior to that of proteins with a molecular weight higher than 40 kDa.

Similar results were found by other researchers. Sitohy *et al.* (12) found that the bacterial protein bands of *Listeria monocytogenes* and *S. Enteritidis* treated with the basic subunits of glycinin and penicillin, respectively, were fainter compared with those of the control. Xing *et al.* (27) reported that oleoyl-chitosan nanoparticles decreased the content of soluble cellular proteins from *E. coli* and *S. aureus* and that the content reduced more significantly as the incubation time with the oleoyl-chitosan nanoparticles increased.

In this study, GBP exhibited a strong antibacterial efficacy against *S. aureus*. AFM directly demonstrated that GBP severely damaged the cell structure of *S. aureus* at the nanoscopic level. Changes in the leakage of β -galactosidase and intracellular substances revealed that GBP could increase the permeability of the bacterial membrane. The destruction of the membrane by GBP decreased the activity of respiratory-chain dehydrogenase on the bacterial cell membrane. This might have inhibited bacterial cell respiration. Furthermore, the destruction of the membrane by GBP hampered the transportation

of bacterial cell nutrients. Consequently, *S. aureus* could not grow and multiply normally and even died because of the inhibition of respiration.

Acknowledgments This study was supported by the National Natural Science Foundation of China (31371839), the Major Plan of Study and Development (2015GSF120006), the Science and Technology Agency, Shandong Province, Science and Technology Development Plan, Shandong Province Colleges and Universities (J13LE02), and the Program for Science and Technology Innovation Team in Universities of Henan Province (16IRTSTHN007).

Disclosure The authors declare no conflict of interest.

References

- Carocho M, Barreiro MF, Morales P, Ferreira IC. Adding molecules to food, pros and cons: A review on synthetic and natural food additives. *Compr. Rev. Food Sci. F.* 13: 377-399 (2014)
- Li YQ, Han Q, Feng JL, Tian WL, Mo HZ. Antibacterial characteristics and mechanisms of ϵ -poly-L-lysine against *Escherichia coli* and *Staphylococcus aureus*. *Food Control* 43: 22-27 (2014)
- Gyawali R, Ibrahim SA. Natural products as antimicrobial agents. *Food Control* 46: 412-429 (2014)
- Tang WT, Yuan HN, Zhang H, Wang L, Qian HF, Qi XG. An antimicrobial peptide screened from casein hydrolyzate by *Saccharomyces cerevisiae* cell membrane affinity method. *Food Control* 50: 413-422 (2015)
- Park CB, Lee JH, Park IY, Kim MS, Kim SC. A novel antimicrobial peptide from the loach, *Misgurnus anguillicaudatus*. *FEBS Lett.* 411: 173-178 (1997)
- Todorov SD, Wachsmann MB, Knoetze H, Meincken M, Dicks LM. An antibacterial and antiviral peptide produced by *Enterococcus mundtii* ST4V isolated from soya beans. *Int. J. Antimicrob. Ag.* 25: 508-513 (2005)
- Oguro Y, Yamazaki H, Takagi M, Takaku H. Antifungal activity of plant defensin AFP1 in *Brassica juncea* involves the recognition of the methyl residue in glucosylceramide of target pathogen *Candida albicans*. *Curr. Genet.* 60: 89-97 (2014)
- Fernandez DI, Le Brun AP, Whitwell TC, Sani MA, James M, Separovic F. The antimicrobial peptide aurein 1.2 disrupts model membranes via the carpet mechanism. *Phys. Chem. Chem. Phys.* 14: 15739-15751 (2012)
- Park SC, Kim JY, Lee JK, Hahn KS, Park Y. Antibacterial action of new antibacterial peptides, Nod1 and Nod2, isolated from *Nordotis discus discus*. *J. Agr. Food Chem.* 60: 6875-6881 (2012)
- Sung WS, Park Y, Choi CH, Hahn KS, Lee DG. Mode of antibacterial action of a signal peptide, Pep²⁷ from *Streptococcus pneumoniae*. *Biochem. Biophys. Res. Co.* 363: 806-810 (2007)
- Hu SD, Liu H, Qiao SY, He PL, Ma X, Lu WQ. Development of immunoaffinity chromatographic method for isolating glycinin (11S) from soybean proteins. *J. Agr. Food Chem.* 61: 4406-4410 (2013)
- Sitohy MZ, Mahgoub SA, Osman AO. *In vitro* and *in situ* antimicrobial action and mechanism of glycinin and its basic subunit. *Int. J. Food Microbiol.* 154: 19-29 (2012)
- Damodaran S, Kinsella JE. Effect of conglycinin on the thermal aggregation of glycinin. *J. Agr. Food Chem.* 30: 812-817 (1982)
- Delwiche SR, Pordesimo LO, Panthee DR, Pantalone VR. Assessing glycinin (11S) and β -conglycinin (7S) fractions of soybean storage protein by near-infrared spectroscopy. *J. Am. Oil Chem. Soc.* 84: 1107-1115 (2007)
- Mo XQ, Zhong ZK, Wang DH, Sun XZ. Soybean glycinin subunits: characterization of physicochemical and adhesion properties. *J. Agr. Food Chem.* 54: 7589-7593 (2006)
- Osman A, El-Didamony G, Sitohy M, Khalifa M, Enan G. Soybean glycinin basic subunit inhibits methicillin resistant-vancomycin intermediate *Staphylococcus aureus* (MRSA-VISA) *in vitro*. *Int. J. Appl. Res. Nat. Prod.* 9: 17-26 (2016)
- Nagano T, Hirotsuka M, Mori H, Kohyama K, Nishinari K. Dynamic viscoelastic study on the gelation of 7S globulin from soybeans. *J. Agr. Food Chem.* 40: 941-944 (1992)
- Ibrahim HR, Sugimoto Y, Aoki T. Ovotransferrin antimicrobial peptide (OTAP-92) kills bacteria through a membrane damage mechanism. *Biochim. Biophys. Acta* 1523: 196-205 (2000)
- Beloti V, Barros MA, De Freitas JC, Nero LA, De Souza JA, Santana EH, Franco

- BDGM. Frequency of 2,3,5-triphenyltetrazolium chloride (TTC) non-reducing bacteria in pasteurized milk. *Rev. Microbiol.* 30: 137-140 (1999)
20. Mohammadzadeh A, Farnia P, Ghazvini K, Behdani M, Rashed T, Ghanaat J. Rapid and low-cost colorimetric method using 2,3,5-triphenyltetrazolium chloride for detection of multidrug-resistant *Mycobacterium tuberculosis*. *J. Med. Microbiol.* 55: 1657-1659 (2006)
 21. Zhou QH, Wu ZB, Cheng SP, He F, Fu GP. Enzymatic activities in constructed wetlands and di-n-butyl phthalate (DBP) biodegradation. *Soil Biol. Biochem.* 37: 1454-1459 (2005)
 22. Klapwuk A, Drent J, Steenvoorden JHAM. A modified procedure for the TTC-dehydrogenase test in activated-sludge. *Water Res.* 8: 121-125 (1974)
 23. Neethirajan S, DiCicco M. Atomic force microscopy study of the antibacterial effect of fosfomycin on methicillin-resistant *Staphylococcus pseudintermedius*. *Appl. Nanosci.* 4: 703-709 (2014)
 24. Nan L, Liu YQ, Lü MQ, Yang K. Study on antibacterial mechanism of copper-bearing austenitic antibacterial stainless steel by atomic force microscopy. *J. Mater. Sci.-Mater. M.* 19: 3057-3062 (2008)
 25. Li A, Lee PY, Ho B, Ding JL, Lim CT. Atomic force microscopy study of the antimicrobial action of Sushi peptides on Gram negative bacteria. *Biochim. Biophys. Acta* 1768: 411-418 (2007)
 26. Jang SA, Kim H, Lee JY, Shin JR, Kim DJ, Cho JH, Kim SC. Mechanism of action and specificity of antimicrobial peptides designed based on buforin IIb. *Peptides* 34: 283-289 (2012)
 27. Xing K, Chen XG, Kong M, Liu CS, Cha DS, Park HJ. Effect of oleoyl-chitosan nanoparticles as a novel antibacterial dispersion system on viability, membrane permeability and cell morphology of *Escherichia coli* and *Staphylococcus aureus*. *Carbohydr. Polym.* 76: 17-22 (2009)
 28. Du W, Sun CL, Liang ZQ, Han YF, Yu, JP. Antibacterial activity of hypocrellin A against *Staphylococcus aureus*. *World J. Microb. Biot.* 28: 3151-3157 (2012)
 29. Li WR, Xie XB, Shi QS, Duan SS, Ouyang YS, Chen YB. Antibacterial effect of silver nanoparticles on *Staphylococcus aureus*. *Biometals* 24:135-141 (2011)
 30. Haraguchi H, Tanimoto K, Tamura Y, Mizutani K, Kinoshita T. Mode of antibacterial action of retrochalcones from *Glycyrrhiza inflata*. *Phytochemistry* 48: 125-129 (1998)
 31. Kubo I, Nihei KI, Tsujimoto K. Antibacterial action of anacardic acids against methicillin-resistant *Staphylococcus aureus* (MRSA). *J. Agr. Food Chem.* 51: 7624-7628 (2003)
 32. Moosavy MH, Basti AA, Misaghi A, Salehi TZ, Abbasifar R, Mousavi HAE, Alipour M, Razavi NE, Gandomi H, Noori N. Effect of *Zataria multiflora* Boiss. essential oil and nisin on *Salmonella typhimurium* and *Staphylococcus aureus* in a food model system and on the bacterial cell membranes. *Food Res. Int.* 41: 1050-1057 (2008)
 33. Tang WT, Zhang H, Wang L, Qian HF. Antimicrobial peptide isolated from ovalbumin hydrolysate by immobilized liposome-binding extraction. *Eur. Food Res. Technol.* 237: 591-600 (2013)
 34. Tang YL, Shi YH, Zhao W, Hao G, Le GW. Discovery of a novel antimicrobial peptide using membrane binding-based approach. *Food Control* 20: 149-156 (2009)