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Interaction of MDpep9, a novel antimicrobial peptide from Chinese traditional edible larvae of housefly, with *Escherichia coli* genomic DNA

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ABSTRACT

Antimicrobial peptides from edible insects may serve as a potentially significant group of food preservatives. In the present work, the mode of action of a novel antimicrobial peptide MDpep9 from Chinese traditional edible housefly larvae was investigated. MDpep9 was shown to bind to bacterial DNA from the results of gel retardation and fluorescence quenching experiments. Further investigations confirmed that MDpep9 could bind with the phosphate group of DNA and intercalate into the base pairs in a helix of DNA or locate in hydrophobic environment of DNA. The previous and present results demonstrated that MDpep9 has dual mechanisms of bactericidal activity: disrupting bacterial cell membranes and binding to bacterial genomic DNA to inhibit cellular functions, ultimately leading to cell death. The results of DNA-binding mode may be contributive in designing new and promising antimicrobial peptides for food preservatives.

1. Introduction

Many peptides of plant and animal origin may have regulatory functions in the human system beyond normal and adequate nutrition, which are defined as "food-derived bioactive peptides" (Hartmann & Meisel, 2007). A wide range of activities of these peptides has been described, including antimicrobial properties (Aslim, Yuksekdag, Sarikaya, & Beyatli, 2005; McCann et al., 2006), blood pressure-lowering (ACE inhibitory) effects (Kodera & Nio, 2006), antithrombotic activities (Chabance et al., 1995), antioxidant activities (Erdmann, Grosser, Schipporeit, & Schroder, 2006; Hernandez-Ledesma, Davalos, Bartolome, & Amigo, 2005), and cytoor immunomodulatory effects (Horiguchi, Horiguchi, & Suzuki, 2005; Meisel, 2005). Thereby, such peptides have enormous potential as ingredients of food additives and pharmaceuticals.

With several desirable properties such as heat-tolerant, relatively broad antimicrobial spectrum, and no cross resistance with antibiotics, antimicrobial peptides (AMPs) may serve as a potentially significant group of food preservatives, especially the "food-derived antimicrobial peptides". For further application and development of AMPs as a potential kind of natural food preservatives, research on the mechanism of AMPs is necessary. However, so far there is not a uniform theory to explain the mechanisms of all AMPs. Some researchers believed AMPs could disrupt the cytoplasmic membrane as the lethal event leading to bacterial cell death through a detergent-like "carpet" mechanism (Gazit, Boman, Boman, & Shai, 1995; Steiner, Andreu, & Merrifield, 1988), or the formation of discrete pores that dissipate ion gradients (Christensen, Fink, Merrifield, & Mauzerall, 1988; Juvvadi, Vunnam, Merrifield, Boman, & Merrifield, 1996; Van Kan, Demel, Van der Bent, & de Kruijff, 2003).

An increasing number of AMPs are being described that act on intracellular targets in bacteria, inhibiting protein or cell–wall synthesis, or interacting with deoxyribonucleic acid (DNA) or ribonucleic acid (RNA). Some researchers found that AMPs could reach to the inner structure of cell through membrane and bind to bacterial DNA with or without disrupting membrane (Brogden, 2005; Pellegrini, Thomas, Wild, Schraner, & von Fellenberg, 2000; Ulvatne, Samuelsen, Haukland, Kramer, & Vorland, 2004; Yonezawa, Kuwahara, Fujii, & Sugiura, 1992). As is known, the regions of DNA involve vital processes, such as gene expression, gene transcription and mutagenesis. Studies on the interactions between AMPs and DNA are very interesting and significant not only in understanding the mechanism of interaction, but also for guiding the design of efficient food preservative. However, until recently, most studies just focus on whether a certain AMP can bind to DNA or not, and the interaction mode between AMPs and DNA were still relatively little known, especially for the AMPs from edible insects.

In our previous paper (Tang, Shi, Zhao, Hao, & Le, 2008), we described a membrane-based method to identify AMPs from housefly larvae and successfully isolated peptide MDpep9 (Lys-Ser-Ser-Ser-Pro-Pro-Met-Asn-His). We found that MDpep9 could insert into cell membranes, leading to loss of cytoplasmic membrane integrity. What does then MDpep9 do inside the cell? Can it act on intracellular targets in bacteria? Recent studies suggested that several

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AMPs could bind to DNA or RNA and inhibited the macromolecular synthesis of the cell (Boman, Agerberth, & Boman, 1993; Yonezawa et al., 1992). PR-39, the proline-rich peptide, is known to stop the synthesis of protein after penetrating the cytoplasm of the cell (Boman et al., 1993). MDpep9 also belongs to Pro-rich peptides and has some common properties with the DNA-binding protein, such as being rich in proline and serine and having positive charged residues (Lys), which suggests that MDpep9 has the potential to interact with bacterial DNA. Therefore, in an attempt to clarify the molecular mechanism of bactericidal activity and explore food preservatives from natural source, the interaction properties exerted by MDpep9 on *Escherichia coli* genomic DNA was investigated in this study.

2. Materials and methods

2.1. Materials

Peptide MDpep9 with a sequence of Lys-Ser-Ser-Ser-Pro-Pro-Met-Asn-His was prepared as described by our previous paper (Tang et al., 2008). 8-Anilino-1-naphthalenesulfonic acid (ANS) was purchased from Sigma (St. Louis, MO). Ethidium bromide (EB) was from Amersco Inc. (Solon, OH, USA). All other reagents were of analytical grade.

2.2. Microorganisms and culture conditions

E. coli ATCC 25922 was kindly provided by Wuxi disease prevention and control center (Wuxi, China) and was grown in Luria–Bertani (LB, Difco, USA) agar slant at 37 °C. The media were sterilised by autoclaving121 °C for 15 min. The strains were maintained as frozen stocks held at −80 °C in Luria–Bertani (LB, Difco, USA) broth containing 20 ml/100 ml glycerol. Throughout the experiments, strains were subcultured every 2 weeks on agar media (LB) to keep the microorganisms viable and kept at 4 °C. Before use in experiments, strains were propagated twice in liquid media (LB) at 37 °C overnight. *E. coli* strains were grown at 37 °C in LB broth at 180 rpm for 12 h. Then bacterial cells were collected by 10 min centrifugation at 8000 rpm at 4 °C (Centrifuge 5804R, Eppendorf), and then washed twice with phosphate-buffered saline (PBS, 10 mmol/l, pH 7.2).

2.3. Extracting E. coli genomic DNA

Extraction of genomic DNA from *E. coli* ATCC 25922 by the CTAB method was performed according to a procedure described previously (Van Soolingen, Hermans, de Haas, Soll, & Van Embden, 1991). The purity of the extracted DNA was evaluated according to the optical density ratio of 260 and 280 nm ($OD_{260}/OD_{280} = 1.96$), which was coincidental with the request of experiment (Amagliani, Giammarini, Omiccioli, Brandi, & Magnani, 2007). The concentration of DNA was determined by measuring the absorbance at 260 nm (UV-2102 PCS, Unico) at room temperature.

2.4. Agarose gel electrophoresis

Agarose gel electrophoresis was carried out as described by Tien, Lafortune, Shareck, and Lacroix (2007) with some modifications. Samples were solubilised in 6 × loading buffer (glycerol 30 ml/100 ml, cyanol xylene 0.25 ml/100 ml, bromophenol blue ml/100 ml) and then separated by migration on an agarose gel containing 0.5 ml/100 ml high melting point agarose (Promega) dissolved in TBE buffer (tris–borate 89 mmol/l, EDTA 2 mmol/l, pH 8.3) in presence of 5 μl ethidium bromide (0.5 μg/ml). Migration was done during 15 h at 50 V using electrophoresis system (mini DNA SUB CELL, Bio-Rad, Mississauga, Canada). The DNA fragments were visualised by UV and photographed.

2.5. Gel retardation assay

DNA and MDpep9 were dissolved in 10 mmol/l tris–HCl buffer (pH 7.2) and then mixed to obtain various DNA/MDpep9 samples with constant DNA concentration (63.1 μmol/l) and increasing MDpep9 concentrations (0.9, 1.8, 9, 18, 90 and 180 μg/ml) for 30 min at room temperature. The effect of MDpep9 on the DNA was analysed by agarose gel electrophoresis. A 2 μl of loading dye was added to 15 μl of the DNA mixture before being loaded into the well of an agarose gel. The loaded mixtures were fractionated by electrophoresis, visualised by UV and photographed.

2.6. Atomic force microscopy (AFM) imaging

DNA and MDpep9 were dissolved in 10 mmol/l tris–HCl buffer (pH 7.2) and then mixed to obtain DNA–MDpep9 complex with 63.1 μmol/l of DNA and 18 μg/ml of MDpep9 for 30 min at room temperature. Prior to AFM measurements, the complex suspension was diluted 10-times with water and 2 μl of the resulting solution was then deposited onto a freshly cleaved mica substrate. Samples were imaged after water evaporation. Experiments were performed with a multimode nanoscope atomic force microscopy (Santa Barbara, United States) operating in tapping mode (TM) (Volcke et al., 2006). All images were recorded in air at room temperature, at a scan speed of 1.0 Hz.

2.7. Absorption titration

DNA and MDpep9 were dissolved in 10 mmol/l tris–HCl buffer (pH 7.2) and then mixed to obtain various DNA/MDpep9 samples with constant DNA concentration (63.1 μmol/l) and increasing MDpep9 concentrations (0, 9, 18 and 36 μg/ml). Transmittance of the mixed solutions was measured in the range of wavelengths 220–320 nm (UV-2102 PCS, Unico). All measurements were made in a quartz cuvette (1 cm in width) at room temperature.

2.8. Fluorescence measurement

The fluorescence spectra of *E. coli* genomic DNA (6.31 μmol/l) in the absence and presence of MDpep9 were measured using 650-60 fluorophotometer (Hitachi, Japan) at room temperature. The excitation was at 535 nm ($\lambda_{ex} = 535$ nm) in this study. Then the change of fluorescence spectra from 550 to 750 nm was measured when MDpep9 with increasing concentrations (0, 9, 18 and 36 μg/ml) were added to DNA with fixed concentration (6.31 μmol/l) at room temperature. Tris–HCl buffer (10 mmol/l, pH 7.2) was used as blank solution for all of the samples.

2.9. Competitive binding of MDpep9 and ethidium bromide (EB) with bacterial DNA

The fluorescence measurements of competitive binding assays were carried out in a tris–HCl buffer (10 mmol/l, pH 7.2) containing fixed concentration of EB (2.54 μmol/l)–DNA (6.31 μmol/l) with varying concentrations of MDpep9 (0, 9, 18 and 36 μg/ml). The EB–DNA solution was placed in the thermostat water-bath at 37 °C for 5 min for equilibrium. Then aliquots of stock solution of MDpep9 were added to the EB–DNA solution and the fluorescence spectra were measured for each test solution after 30 min of incubation at 37 °C. The solutions were excited at 535 nm and spectra were recorded from 550 to 750 nm.

Another assay was used to test the competitive binding of EB against MDpep9 with bacterial DNA as follows. The fluorescence measurements were carried out in a 10 mmol/l tris–HCl buffer (pH 7.2) containing fixed concentration of MDpep9 (18 μg/ml)–DNA (6.31 μmol/l) by varying the concentrations of EB (0, 2.54, 5.08 and 10.08 μmol/l). The MDpep9–DNA solution was placed in the thermostat water-bath at 37 °C for 5 min for equilibrium. Then aliquots of stock solution of EB were added to the MDpep9–DNA solution. The fluorescence spectra were measured for each test solution after 30 min of incubation at 37 °C. The solutions were excited at 535 nm and spectra were recorded from 550 to 750 nm.

2.10. Influence of phosphate anion on the binding of MDpep9 with DNA

Different volumes of aliquots of stock solution of 0.2 mmol/l NaH_2PO_4 (pH 7.2) were added to the EB (2.54 μmol/l)–DNA (6.31 μmol/l)–MDpep9 (18 μg/ml) solution, the final concentrations of which were 3, 6, 12, 24 and 48×10^{-4} mol/l, respectively. And then the fluorescence was measured for each test solution. The solutions were excited at 535 nm and fluorescence intensities were recorded at 603 nm. The relative fluorescence intensity (I_R) was calculated according to the following equation:

$$I_R = I_C/I_{C0}$$

where I_C is the fluorescence intensity of the EB–DNA–MDpep9 system with NaH_2PO_4, and I_{C0} is the fluorescence intensity of EB–DNA system without NaH_2PO_4.

2.11. Statistical analysis

Analysis of variance (ANOVA) was performed using the statistical analysis system (SAS, Version 8.0, 2000, Cary, NC, USA). Error bars were given for a 95% confidence interval. Experiments were triplicated and the means of the three data sets were presented.

3. Results and discussion

3.1. Prediction of DNA-binding activity of MDpep9 using neural networks

Previous studies have focused on the peptide–membrane interaction (Christensen et al., 1988; Gazit et al., 1995; Juvvadi et al., 1996; Steiner et al., 1988; Van Kan et al., 2003), which represent the initial steps of bactericidal process. Though vitally important to bactericidal activity, the mechanism of AMPs–intracellular targets interaction has not yet been completely understood. MDpep9 is rich in proline and serine, and has positive charged residues (Lys), which are common properties of DNA-binding protein, suggesting that MDpep9 may bind to bacterial DNA to play its bactericidal role.

Using neural network based tool, prediction of DNA-binding activity of MDpep9 was performed in the DBS-PRED database (http://gibk26.bse.kyutech.ac.jp/jouhou/shandar/netasa/dbs-pred/) (Ahmad, Gromiha, & Sarai, 2004). The result showed that the predicted probability of MDpep9 to bind with DNA was 100.0%, and binding site predictions were as follows: K1, S2, S3, S4, P5 and P6.

3.2. Gel retardation assay

Agarose gel electrophoresis of free DNA and DNA with increasing amounts of MDpep9 (0.9, 1.8, 9, 18, 90 and 180 μg/ml) is depicted in Fig. 1. As shown in Fig. 1, with the increasing amounts of MDpep9, the migration of DNA through the gel decreased. Upon incubation of the DNA with ⩾9 μg/ml of MDpep9, DNA was partly complexed since part of the DNA remained in the well and slight downward shifts in mobility was observed, suggesting that antimicrobial peptide MDpep9 could bind to bacterial DNA.

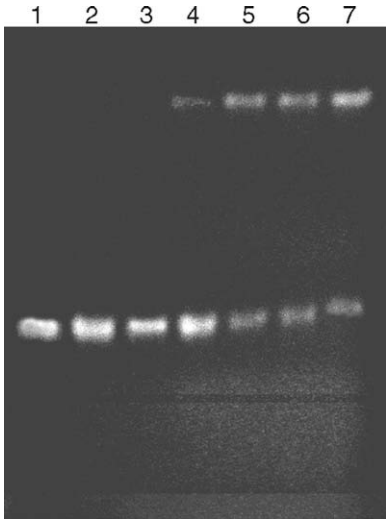

Fig. 1. Agarose gel electrophoresis of free DNA and DNA with increasing amounts of MDpep9. Band 1, free DNA; and Bands 2–7, DNA with increasing amounts of MDpep9 (0.9, 1.8, 9, 18, 90 and 180 μg/ml).

The binding mode of MDpep9 with DNA was further supported by following AFM, ultraviolet and fluorimetric assays, which might illustrate its antimicrobial mechanism.

3.3. AFM imaging

Immobilising DNA is one way to know the interaction with other materials, and the AFM imaging is widely applied to see the immobilised DNA. To investigate binding of *E. coli* DNA to MDpep9, high-resolution images of DNA in the presence of the peptide were obtained using AFM. We performed AFM imaging in the tapping mode (TM-AFM). Representative images of free DNA and AFM image of MDpep9–DNA complexes are shown in Fig. 2. The images showed that DNA in the absence of peptide (Fig. 2a and b) displayed a closed geometry, looking like relaxed circles, with little twisting of the strands. In the presence of 18 μg/ml MDpep9 (Fig. 2c and d), an increase in the number and size of peaks associated along the backbone of the DNA could be seen. This phenomenon indicates that most of DNA molecules can be complexed with the peptide MDpep9. This is in agreement with the results of gel retardation assay.

3.4. Absorption titrations

Electronic absorption spectroscopy is usually utilised to determine the binding of complexes with the DNA. Fig. 3 illustrates that with increasing concentration of MDpep9, the maximum absorption intensity decreased significantly accompanied by a small blue shift of 6 nm (from 260 to 254 nm), due to binding of MDpep9 to the phosphate backbone of DNA double helix. This finding is consistent with the study by Selim, Chowdhury, and Mukherjea (2007). In this work, the pH of all the solutions was about 7.2, and hence DNA appeared as a polyion with considerable negative charges. Many alkalescent amino acids (e.g., Lys and Arg) in AMPs can bind onto the phosphate fragments of DNA, which will reduce charge density in DNA and increase its flexibility, and some chromophores in DNA will sink inby, which diminishes dipole moment (Widom & Baldwin, 1980). As known, 'affinity' through positively charged amino acid (Lys and Arg) side chains interacting with electronegative DNA phosphate backbone plays an important role in

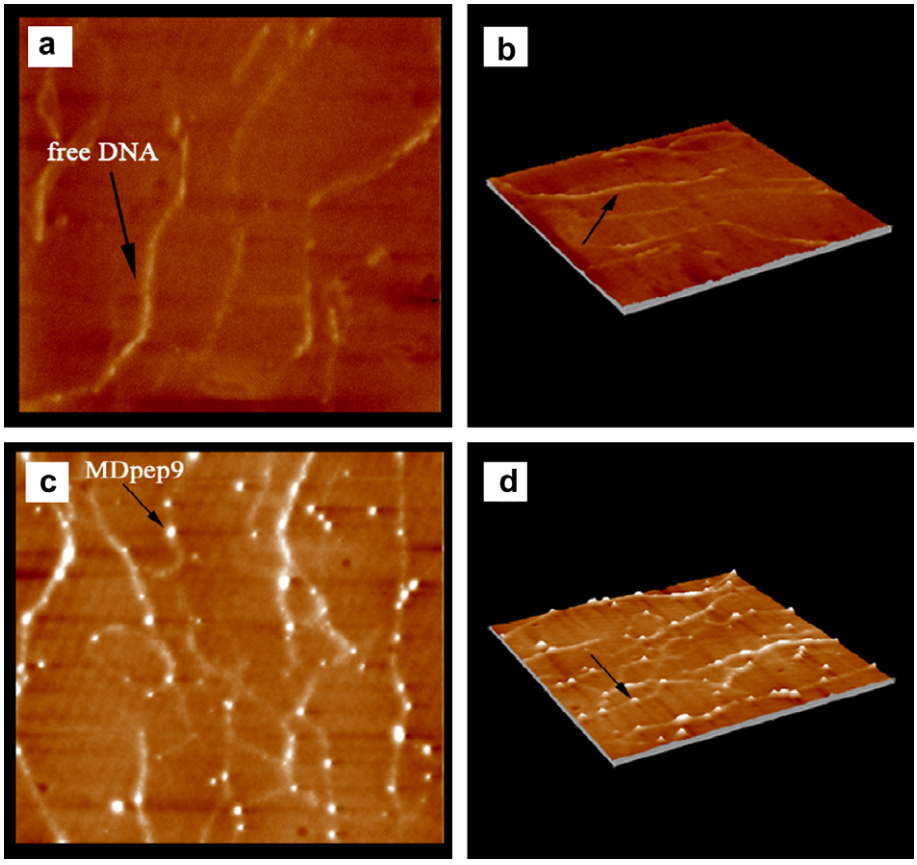

Fig. 2. AFM image of *Escherichia coli* genomic DNA with and without MDpep9. (a) The DNA in the absence of peptide; (b) 3D view in the absence of MDpep9; (c) the peptide–DNA complexes at an MDpep9 concentration of 18 μg/ml; and (d) 3D view in the presence of MDpep9.

binding DNA for proteins (Chen, Wu, & Lim, 2007). Using ExPASy Proteomics tools, prediction of physico-chemical parameters of MDpep9 was performed (http://www.expasy.ch/cgi-bin/protparam). The result showed that the theoretical isoelectric point (pI) of MDpep9 was 8.76 with one positively charged residue (Lys). As known, the thermodynamic dissociation constants (pK_a) for Lys are 2.18 (pK_{a1}), 8.95 (pK_{a2}), and 10.53 (pK_{a3}), respectively. And p*I* value for Lys is 9.74. Therefore, Lys is electropositive at pH 7.2 (tris–HCl buffer), which can bind with DNA phosphate fragments through electrostatic interaction. Thus, the presence of the positively charged residue (Lys) is required for the antimicrobial activity of MDpep9, and the electrostatic interaction between Lys residue and DNA phosphate fragments may be the main factor that underlies DNA-binding activity of MDpep9. Moreover, as Ahmad et al. (2004) claimed, the serine residue was of particular significance, which highly existed in DNA-binding sites of peptides. Thus, three serine residues in MDpep9 might also play an important role in the overall recognition of DNA targets. Therefore, the above results indicated that MDpep9 might bind to the DNA with the joint effect of lysine residue and three serine residues, which was consistent with our prediction of DNA-binding activity of MDpep9 using neural network based tool.

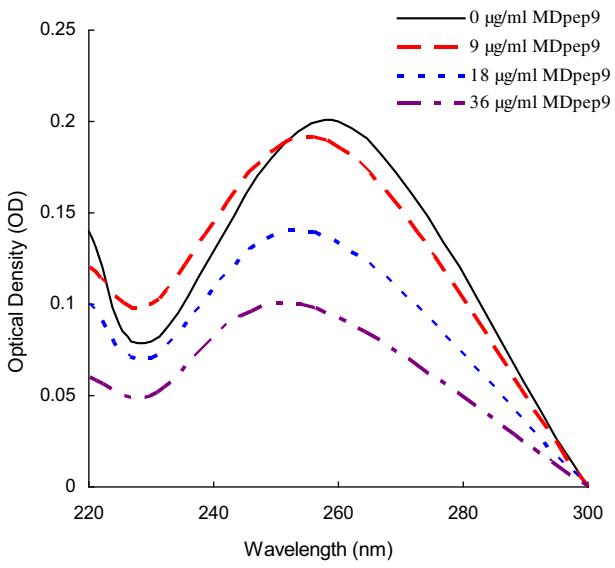

Fig. 3. Ultraviolet spectra of *Escherichia coli* genomic DNA in the presence of increasing amounts of MDpep9. A fixed concentration (63.1 μmol/l) of *E. coli* genomic DNA was treated with increasing amounts of MDpep9 (peptide concentration: (a) 0; (b) 9 μg/ml; (c) 18 μg/ml; and (d) 36 μg/ml). Ultraviolet spectra were measured in the range of wavelengths 220–320 nm. All measurements were made in a quartz cuvette (1 cm in width) at room temperature.

3.5. Fluorescence spectra study

The spectrophotometric titrations of MDpep9 with *E. coli* genomic DNA (0–36 μg/ml) provided more information about the interaction mode of DNA–peptide.

As shown in Fig. 4, in the absence of MDpep9, *E. coli* DNA emitted weak luminescence in tris buffer at ambient temperature, with a maximum appearing about 603 nm excited at 535 nm. Upon addition of MDpep9 into the solution containing the DNA, the fluorescence intensity was dramatically enhanced. The fluorescence signals increased with increasing the concentrations of MDpep9,

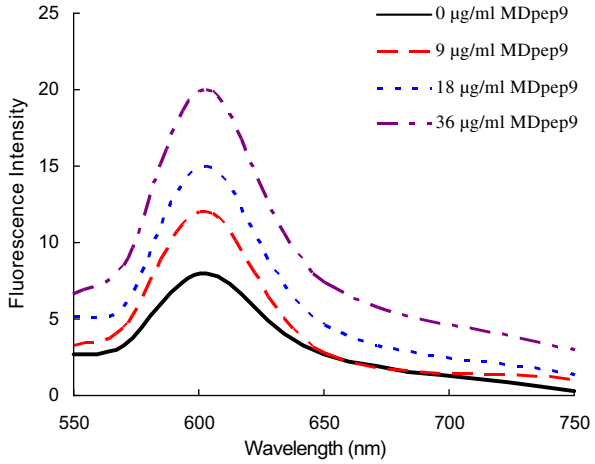

Fig. 4. Fluorescence spectra of *Escherichia coli* genomic DNA in the presence of increasing amounts of MDpep9. A fixed concentration (6.31 μmol/l) of *E. coli* genomic DNA was treated with increasing amounts of MDpep9 (peptide concentration: (a) 0; (b) 9 μg/ml; (c) 18 μg/ml; and (d) 36 μg/ml). Fluorescence spectra were measured from 550 to 750 nm (λ_{ex} = 535 nm).

which might be largely due to the increase of the molecular planarity of DNA and the decrease of the collisional frequency solvent molecules with DNA, being caused by the planar group of MDpep9 stacks between adjacent base pairs of DNA (Nyarko, Hanada, Habib, & Tabata, 2004). The results indicate that MDpep9 could intercalate into the base pairs in a helix of DNA or locate in hydrophobic environment of DNA, and the complex could be stabilised by the stacking interaction with the DNA bases, which led to the increase of the fluorescence intensity of DNA.

3.6. Competitive binding of MDpep9 and EB with bacterial DNA

To further clarify the interaction pattern of MDpep9 with DNA, the competitive binding experiment was carried out using EB as a probe (λ_{ex} = 535 nm and λ_{em} = 550–750 nm). This method can be used for measuring the ability of a compound, having an affinity for DNA, to prevent intercalation of EB to DNA. In general, when small molecules bind to DNA, changes to fluorescence are noted relative to what is observed for solutions without the ligand. However, when a second ligand, which competes for the DNA-binding sites, is added, fluorescence quenching will be observed. EB, a well-known DNA intercalator, emits intense fluorescence in the presence of DNA due to its strong intercalation between the adjacent DNA base pairs. This enhanced fluorescence can be quenched when it coexists with reagent molecule that the similar reaction can occur with, which can be used to monitor the mode of binding thereby indicating the ability of a compound to prevent intercalation of EB to DNA (Lepecq & Paoletti, 1967).

As shown in Fig. 5a, the addition of MDpep9 to DNA pretreated with EB caused obvious fluorescence quenching, indicating that MDpep9 competed with EB in binding to DNA. This observation suggested that some of the EB molecules previously intercalated into the DNA base pairs (a hydrophobic medium) were replaced by MDpep9, and released into the aqueous solution (a hydrophilic medium). Consequently, a decrease in the excitation was observed (Song et al., 2007).

Further support for MDpep9 binding to DNA via intercalation was given through competitive binding assay of EB with bacterial DNA against MDpep9. With the addition of EB, the characteristic fluorescence band of MDpep9–DNA complex rose gradually (Fig. 5b), indicating that some of the EB molecules intercalated into the DNA base pairs instead of MDpep9. EB–DNA replaced MDpep9–DNA gradually.

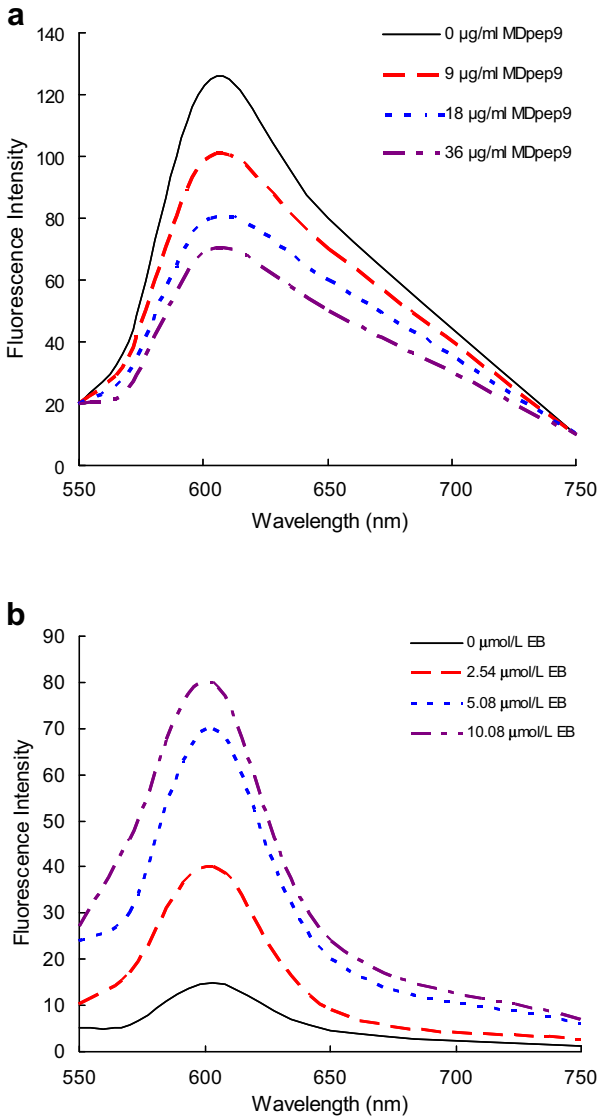

Fig. 5. Competitive binding of MDpep9 and EB with *Escherichia coli* genomic DNA. (a) Fluorescence spectra of DNA–EB in the presence of increasing amounts of MDpep9. A fixed concentration of DNA (6.31 μmol/l)–EB (2.54 μmol/l) was treated with increasing amounts of MDpep9 (peptide concentration: (a) 0; (b) 9 μg/ml; (c) 18 μg/ml; and (d) 36 μg/ml). (b) Fluorescence spectra of DNA–MDpep9 in the presence of increasing amounts of EB. A fixed concentration of DNA (6.31 μmol/l)–MDpep9 (18 μg/ml) was treated with increasing concentrations of EB (EB concentration: (a) 0; (b) 2.54 μmol/l; (c) 5.08 μmol/l; and (d) 10.08 μmol/l). The solutions were excited at 535 nm and spectra were recorded from 550 to 750 nm.

The competitive binding assays showed that there was a state of equilibrium between two complex systems, MDpep9–DNA and EB–DNA:

$$\text{EB—DNA} + \text{MDpep9} \leftrightarrow \text{MDpep9—DNA} + \text{EB}$$

3.7. Influence of phosphate anion on the competitive binding of MDpep9 and EB with DNA

The addition of MDpep9 to EB–DNA system decreased the fluorescence strength as above, but when phosphate was added to EB–DNA–MDpep9 complex, the fluorescence intensity increased significantly again (data not shown).

Previous researches showed that protein binding sites as well as the binding strength varied accordingly with the conformational change upon binding, where solvent, pH and other factors were

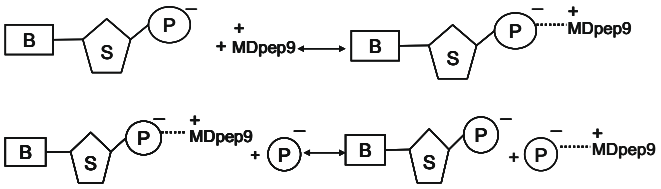

Fig. 6. Schematic representation of the effect of phosphate on the binding of MDpep9 with DNA (B, bases; P, phosphate group; and S, deoxyribose sugar group).

included. Therefore, compared to the binding conformation, protein non-binding sites were suboptimally stable (Chen & Lim, 2008). The addition of phosphate weakened the quenching of MDpep9 to the fluorescence of EB–DNA, suggesting that the phosphate anion from phosphate instead of that from DNA could interact with MDpep9 through electrostatic interaction. In other words, as DNA trends to form the energetically stable conformation (unbinding conformation), with the addition of phosphate, MDpep9–DNA complex (energetically unstable conformation) was dissociated into unbinding DNA (energetically stable conformation) (Fig. 6). All the results suggested that phosphate anion in DNA double helix was one of binding sites, which was consistent with the result of ultraviolet spectra study.

4. Conclusions

Previous studies focused on the peptide–membrane interaction which represents the initial steps of bactericidal process. However, little is known about the peptide–intracellular targets interaction. Here we reported that a novel antimicrobial peptide MDpep9 derived from Chinese traditional edible larvae of housefly showed bactericidal activity. Our current and previous studies about MDpep9 suggested its dual antimicrobial mechanisms: disrupting cell membrane and binding to bacterial genomic DNA to inhibit cellular functions. In DNA-binding process, MDpep9 could bind to the phosphate anion in DNA double helix through electrostatic interaction firstly, and intercalate the groove in DNA double helix, which might affect the replication, transcription and expression to repress even led to cell death. Although a detailed experiment on the interaction between MDpep9 and nucleic acids in vivo is needed, the present results lead us to believe that MDpep9 inhibits the cellular functions by binding to DNA of cells after penetrating the cell membranes, resulting in the rapid cell death. The results of DNA-binding mode may be contributive in designing new and promising AMPs for a variety of food preservatives.

Acknowledgements

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