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Synthesis and Antibacterial Evaluation of Liposomal Particles Containing the Recombinant Peptide rCAP18

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Introduction

Antimicrobial peptides (AMPs) have been widely investigated as potential bio-antibiotics against bacteria. AMPs are natural antimicrobial agents that make up the innate immune system. These peptides mainly target the anionic regions in the microbial membranes due to their cationic charge, which causes the initial interaction between the peptide and the microbial membrane. Subsequently, the hydrophobic amino acids of the peptide penetrate into the hydrophobic core of the membrane and cause the loss of membrane function (Cantor et al., 2019; Ciumac et al., 2019; Sato & Feix, 2006). AMPs are considered as alternative compounds to kill bacteria because of their biocompatibility, biodegradability, and strong bactericidal effects. However, their use as food preservatives is limited due to their sensitivity to enzymatic degradation and their potential interaction with food components, such as fats and proteins (Cantor et al., 2019; Gomaa et al., 2017). Encapsulating peptides in liposomes may be a suitable method to solve such problems (Alipour et al., 2008; Mosquera et al., 2014; Ron-Doitch et al., 2016). So far, various forms of encapsulation, such as liposomes or films, have been used for the controlled release of antimicrobial peptides. The obtained results have shown that the encapsulation of peptides by liposomes is an effective strategy for establishing the stability and protecting the antimicrobial activity of AMPs (Cantor et al., 2019; da Silva Malheiros et al., 2010; Gomaa et al., 2017; Millette

et al., 2007). The goal of this study was to develop and produce phosphatidylcholine (PC) liposome containing rCAP18, characterize the produced vesicles, and measure their antibacterial activity.

Material and Methods

We used recombinant CAP18 (rCAP18) as an antibacterial peptide. For protein production, Pichia pastoris strain X-33 containing the recombinant cap18 gene that was created in our previous study was used. After the production and purification of recombinant peptides, encapsulation was performed inside the liposomes containing PC. The efficiency of peptide entrapment in liposomes was measured. Morphological examination of Lipo@rCAP18 nanovesicles was performed using transmission electron microscopy (TEM). Dynamic light scattering (DLS) method was used to measure the size of the synthesized liposomes. Finally, the antibacterial activity of the free and encapsulated rCAP18 against *Escherichia coli*, *Pseudomonas aeruginosa*, *Xanthomonas citri* subsp. *citri* and *Staphylococcus aureus* was evaluated and compared based on the MIC and MBC values.

Result and Discussion

Our results showed that the structures of Lipo@rCAP18 are spherical and single-layered. The formation of liposomal structures was confirmed by the analysis of surface functional groups in the graphs of Lipo@control and Lipo@rCAP18. The entrapment rate of rCAP18 peptides inside the liposome was 60 %. According to the study conducted in 2003 by Were et al., it was determined that the concentration of compounds trapped in liposomes is related to the type of lipid compounds used and the electrostatic and hydrophobic interactions between antimicrobial peptides and phospholipids (Were et al., 2003). Peptide release from liposomes also depends on the type of amino acids that form the antibacterial peptide. Our results showed that the release rate of rCAP18 from liposomes was approximately 54 % after 8 months. It appears that the type of phospholipid forming the liposome played a critical role in the slow release of the peptide. This result agrees with the results of Taylor et al., who showed that the composition of phospholipids forming vesicles has an important effect on the release of cationic peptides. (Taylor et al., 2008). Evaluation of the antibacterial activity of rCAP18 and Lipo@rCAP18 showed that the minimum inhibitory concentration of free rCAP18 for *E. coli*, *P. aeruginosa*, *X. citri* and *S. aureus* strains was determined to be 135, 101, 80, and > 320 µg/ml. MIC value of rCAP18 enclosed in the liposome was >320, 135, 180, and 320 µg/ml, for the strains mentioned above, respectively. The comparison of MIC results showed that free and encapsulated forms of rCAP18 have a more growth-inhibitory effect against *P. aeruginosa* and *X. citri* strains.

Conclusions

Considering that the structure of liposomes is similar to the biological membranes, they can be easily attached to the bacterial membranes and interact with them. Therefore, liposomes are

considered carriers of antimicrobial peptides and have become safe and effective products in food and cosmetics. In addition, encapsulating antibacterial peptides inside liposomes maintains their stability against environmental factors.

Keywords: Antibacterial property, phosphatidylcholine, Liposome, Recombinant CAP18.

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Declaration of conflict of interest

The authors of this study declare that they have no conflicts of interest.