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Effects of vesicle components on the electro-permeability of lipid bilayers of vesicles induced by pulsed electric fields (PEF) treatment

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ABSTRACT

In this study, the effects of pulsed electric field (PEF) treatments on the electro-permeability of vesicles made from dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), dioleoyl-sn-glycero-3-phosphocholine (DOPC), Soya phosphatidylcholine (soya PC), cholesterol and a binary mixture of them encapsulated 5(6)-Carboxyfluorescein (5(6)-CF) were investigated, based on the electric field strength of 0–40 kV/cm and treatment time of 0–4.8 ms. Results indicated that the electro-permeability of lipid membrane was directly related to the composition of lipid bilayers membrane of vesicles. As compared to DPPC vesicles, DOPC vesicles were much more sensitive to PEF treatment. For the DOPC vesicles, the 5(6)-CF release percentage (R) was 9.24%, 20.12%, and 30.62% after PEF treatment at 20, 30, and 40 kV/cm for 4.8 ms treatment time, respectively. However, for the DPPC vesicles, it was only 1.84%, 4.02%, and 6.51%, respectively. On the other hand, the electro-permeability of DOPC vesicles was greatly affected by the addition of DPPC. The electro-permeability of DOPC vesicles was decreased sharply with the increase of DPPC molar ratio. Under the PEF treatment with 40 kV/cm and 1.2 ms, it was observed that the R of DOPC vesicles decreased markedly from 27.56% to 9.80% with the molar ratio of DPPC increased from 0 to 40%.For the soya PC vesicles, the R was dropped sharply from 48.13% to 24.11% with the molar ratio of cholesterol increased from 0 to 50%. Raman spectroscopy analysis revealed that the decrease of electro-permeability of soya PC vesicles with the addition of cholesterol was responsible for the incorporating of cholesterol into hydrocarbon partition of lipid bilayers, leading to the order arrangement of hydrocarbon chain of phospholipids, which was resulted an increase in the thickness of bilayers membrane and decrease in the fluidity of bilayers membrane. However, for the mixture of DOPC and DPPC (DOPC/DPPC) vesicles, there has no effect on the conformation of hydrocarbon chain of phospholipids and the fluidity of bilayer membrane of DOPC/DPPC vesicles with the addition of DPPC.

1. Introduction

Recently, pulsed electric fields (PEF) technology has been widely studied for many operations in the food and bioengineering industries (Bermudez-Aguirre et al., 2011; Han et al., 2012a,b), such as the sterilization of microorganisms (Saldana et al., 2010; Bermudez-Aguirre et al., 2012), and the improvement of mass transfer in plant or animal cells and others (Delsart et al., 2012; Loginova et al., 2011). With the aspect of food preservation, PEF treatment as a promising non-thermal food processing and preservation technology has received much attention (Leong et al., 2015 2016;, Suwandy et al., 2015; Bekhit et al., 2014; Sampedro et al., 2014; Huang et al., 2014; Guo et al., 2014; Mhemdi et al., 2014),

List of symbols and abbreviations: PEF, Pulsed electric fields; Soya PC, Soya phosphatidylcholine; DPPC, Dipalmitoyl-sn-glycero-3-phosphocholine; DOPC, Pioleoyl-sn-glycero-3-phosphocholine; DOPC/DPPC, Mixture of DOPC and DPPC; CF, 5 (6)-carboxyfluorescein; R (%), 5 (6)-CF release percentage; T_m, Phase transition temperature; PCH, Percentage of molar rate of cholesterol.

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and become one of the most potential food preservation technologies alternate to traditional thermal treatments such as cooling (Sun and Wang, 2000; Desmond et al., 2000; Zheng and Sun, 2004; Wang and Sun, 2002a, 2002b, 2004), freezing (Kiani and Sun, 2011) and drying (Delgado and Sun, 2002; Cui et al., 2008).

A variety of microorganism species have been successfully killed by PEF treatments in various liquid foods (Saldana et al., 2010; Fernandez-Molina et al., 2006; Walkling-Ribeiro et al., 2009). The mechanism of PEF processing to inactivate microorganisms could be explained as a combination of electroporation and electropermeabilization (Teissie et al., 2005; Raffy et al., 2004). Previous studies have shown that the cell death induced by PEF was directly related to the distribution of the integrity of cell plasma membrane and the change of its permeability (Weaver and Chizmadzhev, 1996; Hamilton and Sale, 1967; Tekle et al., 2001). When a transmembrane potential reaches a critical value of approximately 0.2–1 V for a bimolecular lipid which might lead to the breakdown of membrane (Kinosita et al., 1988), and the hydrophilic reversible or irreversible pore could be generated. The irreversible pores cause the death of microorganism. Molecular dynamics simulations have demonstrated that the formation of a pore by PEF includes three steps. Firstly, building the transmembrane potential up by the applied external electric field, causing water defects in the bilayer membrane. Then, a water file formed by water molecules spans the bilayers membrane by establishing hydrogen bonds with each other. Finally, molecular rearrangement of the phospholipids in the vicinity of this water defect occurs and phospholipid molecules move towards this water channel to give a hydrophilic pore lined with phospholipid head groups (Vernier et al., 2013; Ziegler and Vernier, 2008).

The influence of various process parameters and product properties on the efficiency of microbial inactivation by PEF treatment has been extensively studied (Bermudez-Aguirre et al., 2012; Álvarez et al., 2003). The results indicated that the efficiency of PEF treatment depended on field parameters and product properties. It has been demonstrated that the main process parameters, such as electric field strength, number of pulses, and temperature; product properties, such as conductivity, pH were the key factors for determining the microbial lethal efficiency of PEF treatment (Evrendilek and Zhang, 2005; Álvarez et al., 2000). However, microbial inactivation efficiency of PEF treatment also depends on the type of microorganism and species, or even strains and their growth phase (Saldana et al., 2010). For example, gram-positive bacteria were found to be less sensitive to electric pulse treatment than gram-negative bacteria. The bacteria cells from the logarithmic growth phase were killed in markedly higher percentage than cells harvested from the stationary growth phase (Hülsheger et al., 1983). Therefore, process parameters and product properties are not the only the factors that affect the efficiency of PEF treatment; membrane characteristics and cell wall construction are the other factors that should be considered. Contrary to the extensive study of process parameters and product properties, the studies about the influence of the composition of cell membrane on the efficiency of PEF treatment are still rare.

PEF treatment as a non-thermal processing technology can kill bacteria effectively in liquid food. However, the efficiency of PEF treatment is different for various microorganisms or even for the same kind of microorganism at different cultivation environment and growth phase. Previously, Álvarez et al. (2002) reported that *Listeria monocytogenes* cultivated at 4 °C were more sensitive to PEF treatment than the cells cultivated at 35 °C independent of the growth phase. *Escherichia coli* cells cultivated at 20 °C were more easily sterilized by PEF treatment than *E. coli* cultivated at 37 °C (Ohshima et al., 2002). On the other hand, *Staphylococcus aureus* exposed to heat (45 °C) and alkaline (pH = 9.5) shocks increased the resistance to PEF (Cebrián et al., 2012). The reason for this phenomenon may ascribe to the changes of fatty acyl chain content in microbial cell membrane under lower growth temperature. It is well recognized that microorganisms can adjust their membrane lipid composition in response to changes in growth temperature, pH and osmotic stress to ensure membrane function such as enzyme activity and solute transportation (Schoug et al., 2008; Beales, 2004). It was reported that *Clostridium botulinum* exhibited an increased level of unsaturation degree in the composition of fatty acid chain of cell membrane, from 27% to 40%, after a reduction in cultivation temperature from 37 °C to 8 °C (Russell et al., 1995). In order to adapt to low temperatures, the composition of fatty acid chain of cell membrane in *E. coli ML30* (Marr and Ingraham, 1962), and *Lactobacillus plantarum* (Russell et al., 1995) changed by synthesizing increased the proportions of unsaturated fatty acids with the expense of saturated fatty acids. The membrane composition of acid-adapted *E. coli* is of more cyclopropane derivatives and fatty acids (Berry and Foegeding, 1997). Therefore, in order to reveal the underlying mechanism that the bacteria resist to PEF treatment, the study about the influence of the composition of cell membrane on the efficiency of PEF treatment is of great importance.

Vesicles constituted by synthetic phospholipids provide reliable models to simulate biomembrane models and they are suitable for systematic investigation on the impact of PEF on lipid bilayers structure. Different types of vesicles have been used to access membrane electro-permeability. First experiments have performed on small unilamellar vesicles with the size 15–30 nm encapsulated with radioactive sucrose more than 30 years ago (Teissie and Tsong, 1981; Raffy and Teissie, 1995). In recent year, giant unilamellar vesicles with the size 10–40 μm which can be directly observed with optical microscopy were developed. Visualization of electropermeability of vesicles has been performed to elucidate the response of the cell membrane to PEF treatments (Riske and Dimova, 2005; Raffy and Teissié, 1997). In present study, large unilamellar vesicles with the size of 1 - 2μmareand match well with the size of microorganism.

The purpose of this study was to obtain a better insight into the influence of the molecular properties and the composition of the cell membrane on the electro-permeability of lipid bilayers membrane. Vesicles made from DOPC, DPPC, soya PC, cholesterol and a binary mixture of them encapsulated with 5(6)-CF were used. The 5(6)-CF release percentage (R value) was used as the indicator of the electro-permeability of lipid bilayers membrane affected by PEF treatment. The relationship between the component of lipid bilayers and the permeability of vesicles induced by PEF treatments were established.

2. Materials and methods

2.1. Materials

Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC, purity 99%) and dioleoyl-sn-glycero-3- phosphocholine (DOPC, purity 99%) were purchased from Corden Pharma Co. (Liestal, Switzerland). Soya phosphatidylcholine (soya PC) (the content of PC > 98%), 5(6)-Carboxyfluorescein (5(6)-CF) and cholesterol were obtained from Aladdin Chemistry Co. Ltd. (Shanghai, China). Chloroform, diethyl ether and diisopropyl ether were obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). All other chemicals used in this study were of reagent grade.

2.2. Liposome preparation

Liposome was prepared from DOPC by reverse phase