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# Influence of resveratrol on interactions between negatively charged DPPC/DPPG membranes and positively charged poly-L-lysine

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ABSTRACT

Resveratrol (Res), a natural polyphenol present in different plants and vegetables, exhibits potential therapeutic activity with cardioprotective, antineurodegenerative, antioxidant, and antitumor action. In this study, the effect of Res on the mutual interactions between positively charged poly-L-lysine (PLL) and negatively charged dipalmitoylphosphatidylcholine/dipalmitoylphosphatidylglycerol (DPPC/DPPG) membranes was studied using Fourier-transform infrared (FTIR) spectroscopy supported by principal component analysis (PCA). The interactions between PLL and DPPC/DPPG membranes were strongly affected by the presence of Res molecules. Depending on the Res concentration and method of its supply (through the water or lipid phase) to the studied peptide–membrane systems, the membrane-induced transition of PLL from an α-helix to an extended left-handed polyproline II helix (PPII) occurred at different temperatures, with different cooperativity, or was even completely suppressed. The influence of PLL on the conformational (*trans/gauche*) state of the hydrocarbon chain region of the lipid membranes and the hydration state of the polar/apolar membrane interface was also modulated by Res, depending on the membrane phase state.

## 1. Introduction

Binding of proteins at lipid membrane surfaces plays an important role in the biological functions of both protein and membrane components. Many protein–membrane interactions have electrostatic character. For this reason, membrane-binding proteins frequently have a fragment of peptide chain that is rich in positively charged amino acids, such as lysine, arginine, and histidine (Murray et al., 1997; Kim et al., 1991; Heimburg et al., 1999; Wang et al., 2004). Antimicrobial agents, which react with bacterial cells through membranes, are typically short peptides rich in positively charged amino acids (Dufourcq et al., 1981; Ben-Tal et al., 1996, 1997; Murray et al., 1999, 2002). It has been shown that ATPases and other membrane-associated transport proteins and enzymes interact with negatively charged lipids (George et al., 1989; Xia and Dowhan, 1995; Yamazaki et al., 1998). In all of these cases, proteins/peptides can bind through electrostatic attraction to the negatively charged membranes.

In contrast to the complex structures of naturally occurring proteins and membranes, simplified models of proteins and membranes are especially useful for systematic studies of the relationships between structures and binding features. Poly-L-lysine (PLL), because of its high positive charge and ability to adopt all of the common secondary structures of proteins (Cieślik-Boczula, 2017a,b; Cieślik-Boczula and Rospenk, 2018), is frequently used as a good model system for positively charged proteins to study electrostatic mutual interactions with negatively charged lipid membranes (Schwieger and Blume, 2007). Meanwhile, phosphatidylglycerol lipids are one of the most common types of anionic lipids found in natural biological membranes, and are often the only type of anionic lipid in prokaryotic cell membranes (Goldfine, 1982). Phosphatidylglycerol molecules are the most frequently used anionic lipids to prepare model lipid membranes with negatively charged surfaces.

In the present paper, dipalmitoylphosphatidylcholine/dipalmitoylphosphatidylglycerol (DPPC/DPPG, 50/50 mol%) membranes are used as a model of a biomembrane with a stable bilayer structure and negatively charged surface, and PLL peptides are used as a model of positively charged basic proteins. It should be mentioned that even though DPPC/DPPG membranes have been widely used as a model of negatively charged biological membranes, they do not perfectly mimic all the features of biomembranes. Using Fourier transform infrared (FTIR) spectroscopy supported by principal component analysis (PCA), the mutual interactions between PLL and DPPC/DPPG membranes as a function of increasing temperature are studied.

The search for chemical compounds that can effectively regulate the interactions between charged proteins and membranes is an interesting task for many researchers in the context of identifying new potentially useful drugs. Additionally, the interactions between these compounds and protein–membrane systems are very often important to elucidate

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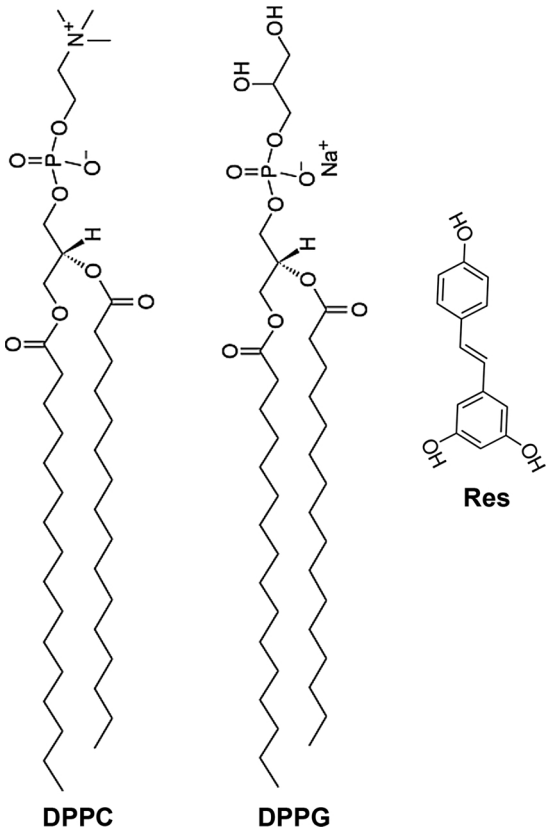


**Fig. 1.** Molecular structures of dipalmitoylphosphatidylcholine (DPPC), dipalmitoylphosphatidylglycerol (DPPG), and resveratrol (Res).

the mechanism of organic compounds with biological activity. Fig. 1 shows the structure of resveratrol (Res), which is a natural polyphenol compound present in red grapes (therefore, also in red wine), other highly pigmented fruits, and vegetables (Catalgol et al., 2012; Tsai et al., 2017). Res has attracted the attention of researchers because of its diverse and beneficial effects on human health (Catalgol et al., 2012; Tsai et al., 2017). The mechanism by which Res exerts its antioxidant, hematoprotective, antineurodegenerative, and antitumor actions has not yet been fully elucidated. It is postulated that the cellular targets of Res molecules are different proteins and biomembranes (Catalgol et al., 2012; Tsai et al., 2017). To determine the effect of Res on the interactions between positively charged basic proteins and anionic lipid membranes, here the DPPC/DPPG/PLL system in the presence of different concentrations of Res supplied by different methods is studied. The roles of Res in the membrane-induced changes of the secondary structure of PLL and in PLL-induced changes of the lipid membranes accompanied by *trans* to *gauche* isomerization of $CH_2$ groups of the lipid hydrocarbon chains and an increase in the hydration of the interface region of DPPC/DPPG membranes are discussed. The conformational (*trans/gauche*) and hydration states of DPPC/DPPG/PLL/Res systems in gel and liquid-crystalline phases are compared. In summary, Res incorporates into the peptide-membrane systems can affect theirs structures, which is of importance when trying to explain the different biological activities or Res molecules.

## 2. Experimental

### 2.1. Materials

1,2-Dipalmitoyl-sn-*glycero*-3-phosphocholine (DPPC) and 1,2-dipalmitoyl-sn-*glycero*-3-phospho-(1′-rac-glycerol) (sodium salt) (DPPG) with purities of > 99.8% were obtained from Sigma-Aldrich, Germany. Res (3,4′,5′-trihidroxy-*trans*-stilbene) with a purity of > 99% and PLL with a molecular weight of approximately 250 kDa were purchased from Sigma-Aldrich, Poland. All compounds were used without further purification. Stock solutions of Res in dimethyl sulfoxide (DMSO) with concentrations of 73 and 183 mg/mL were prepared. PLL was dissolved in deuterium oxide ($D_2O$) with 110 mM sodium chloride (NaCl) at a concentration of 20 mg/mL.

### 2.2. Preparation of DPPC/DPPG liposomes mixed with PLL and Res

DPPC (10 mg) and DPPG (10 mg) were mixed together in a methanol/chloroform (1:1, v/v) solution and then dried under a stream of nitrogen and stored in a vacuum for 4 h. The lipids were dispersed in $D_2O$ (1 mL) with 110 mM NaCl at a higher temperature than the temperature of the main phase transition during ten heating–cooling cycles. Large unilamellar vesicles (LUV) were prepared by extruding the liposomal suspension through a polycarbonate filter containing pores with a diameter of 100 nm (LiposoFast, Avestin, Canada). The PLL-doped DPPC/DPPG membranes were prepared by mixing the liposomal dispersion (40 μL) with the separately prepared stock solution (20 μL) of PLL in $D_2O$ with 110 mM NaCl on a $CaF_2$ window just before FTIR spectroscopic measurement. Res molecules were supplied to the studied systems in two ways. In the first method, Res was supplied through the water phase by the simple addition of the Res stock solution (5 μL) in DMSO to the aqueous solution of PLL (250 μL). In the next step, the PLL/Res mixture (20 μL) was mixed with the liposome suspension (40 μL). In the second method, Res was supplied through the lipid phase. In this case, an appropriate amount of Res was added to the methanol/chloroform solution of DPPC and DPPG lipids, and then the liposomes were prepared according to the previously described procedure. The lipid concentration after liposome preparation was measured using inductively coupled plasma–optical emission spectrometry. The total amount of lipid phosphate was determined using an ARL 3410 spectrometer operated at 213 nm (Cieślik-Boczula et al., 2014). The final amount of DPPC in the prepared liposomes was 91–94% of the theoretical amount. The proportion of Res and/or PLL molecules was adjusted relative to the real number of lipid molecules in the liposomes.

### 2.3. FTIR spectroscopic measurements supported by PCA calculations

A Nicolet Magma 860 FTIR spectrometer was used to collect the FTIR spectra of samples as a function of increasing temperature in the range of 10 to 65 °C. Each sample was incubated at each investigated temperature for 0.5 h using a water bath circulator (Julabo Labortechnik GMbH) before FTIR spectral measurement. Each sample was placed between two $CaF_2$ windows separated by a 56-μm Teflon spacer and measured with 128 scans at a resolution of 2 $cm^{-1}$. To decrease the influence of the signals of Res and/or PLL molecules on the positions of the $\nu CH_2$ bands of lipid membranes, the spectrum of the relevant additives in the region of 3000–2800 $cm^{-1}$ was subtracted from the spectrum of the Res- and/or PLL-doped DPPC/DPPG membranes collected at the same temperature. Before PCA calculations, the spectrum of $D_2O$ with 110 mM NaCl recorded at the same temperature was subtracted from each sample spectrum; a linear function for the baseline correction was applied; normalization to a constant total area in the region of amide I' vibrations, stretching vibrations of C═O groups of lipids, and stretching vibrations of lipid $CH_2$ groups was carried out; and a mean-centering procedure was performed. The software used for pretreatment processes and PCA calculations has been described previously (Cieślik-Boczula, 2017a). The PCA generated both score and loading functions. The differences between the score values of samples were proportional to the differences in the FTIR spectra of samples measured at different temperatures. Thus, the plot of score against increasing temperature describes the character and direction of spectral evolution during the main lipid phase transition. The plot of loading as a function of wavenumber shows the wavenumbers at which