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Feruloyl glycerol and 1,3-diferuloyl glycerol antioxidant behavior in phospholipid vesicles

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ABSTRACT

Feruloyl-*sn*-glycerol (FG) and 1,3-diferuloyl-*sn*-glycerol (F_2G), the by-product of biocatalytic trans-esterification soybean oil and ethyl ferulate, were examined for their behavior in phospholipid vesicles. Based on absorbance and fluorescence methods, FG and F_2G both were found to partition into vesicles and incorporate well into 1,2-dioleoylphosphocholine (DOPC) vesicles. FG and F_2G incorporation resulted in vesicles that were as or slightly more stable than the unloaded vesicles. FG and F_2G both demonstrated the ability to maintain antioxidant properties within the lipid bilayer. Bilayer depth analysis was conducted using the parallax method and molecular modeling.

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1. Introduction

Cinnamic acid derivatives are found to serve in different capacities throughout the plant kingdom. For instance, cinnamic acid esters are found in the waxy surfaces of leaves and as components of the cellulose in plant cell walls. One cinnamic acid derivative in particular, 4-hydroxy-3-methoxy cinnamic acid (ferulic acid), was discovered to absorb UV-light in cell walls of plant spores and pollen (Rozema et al., 2001). The UV-absorbing and antioxidant ability of cinnamates and their derivatives make these molecules attractive for commercial exploitation.

Thanks to the UV-absorbing properties of ferulic acid, there has been considerable interest in synthesizing ferulic acid derivatives on a large scale for decades. Saija et al. (1999) suggested that ferulic and caffeic acids could be used as potential protective agents against photooxidative skin damage. For example, several groups synthesized mono- and di-feruloyl acylglycerols (Guyot et al., 1997; Laszlo and Compton, 2006; Laszlo et al., 2003; Stamatis et al., 1999; Sun et al., 2009; Taniguchi et al., 1999a,b) and Compton et al. (2000) were able to enzymatically generate ferulate esters. More interestingly, it has been demonstrated (Kikuzaki et al., 2002; Compton et al., 2007) that it is possible to successfully produce lipid soluble feruloyl soy glycerols in topical formulations as well (Batovska et al., 2005; Holser, 2008; Holser et al., 2008; Sun et al., 2007) to successfully esterified ferulic acid to glycerol.

1-Feruloyl-*sn*-glycerol (FG) and 1,3-diferuloyl-*sn*-glycerol (F_2G), which are found throughout the plant kingdom (Cooper et al., 1978; Graca and Pereira, 2000), have potential application as active antioxidant ingredients in the cosmetic and food industries. Extraction of FG and F_2G from natural sources, however, has produced limited quantities (Compton and Laszlo, 2009; Lapierre et al., 2001). Consequently, large scale (kg) production of these products was developed (Compton and Laszlo, 2009; Laszlo et al., 2003). Previous work also demonstrated the antioxidant capacity of FG and F_2G (Compton et al., 2012; Kikugawa et al., 2012). It is not clear, however, if formulations for cosmetic and/or food industries would accommodate each molecular species well. One method of incorporating small molecules for potential nutritional and cosmetic applications is to incorporate them into vesicles. However, it was shown that phenolic phytochemicals can alter bilayer properties (Ingólfsson et al., 2014). Thus, this work was undertaken to evaluate the antioxidant performance of FG and F_2G incorporated into 1,2-dioleoylphosphocholine vesicles.

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2. Materials and methods

2.1. Materials

Mike's Brand 100% soybean oil was purchased from Columbus Foods (Chicago, IL) and used as obtained. Ethyl 4-hydroxy-3-methoxy cinnamic acid (ethyl ferulate) was purchased from Ash Chemical Co. (Wuxi City, Jiangsu Province, China). Novozym 435 (*Candida antarctica* lipase B immobilized on acrylic beads) was purchased from Novozymes North America (Franklinton, NC). Linolenic acid was purchased from Nu-Chek-Prep (Elysian, MN). Glycerol (spectroscopic grade), 2-methyl-2-butanol, Tween-20 (not stabilized), 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH), butylated hydroxytoluene (BHT), thiobarbituric acid (TBA), 1,1,3,3-tetramethoxypropane, ferulic acid (4-hydroxy-3-methoxy cinnamic acid) and 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) were purchased from Sigma–Aldrich Co. (St. Louis, MO). Solvents were HPLC grade, purchased from Sigma–Aldrich Co., and used without further purification. 1,2-Dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) was purchased from Avanti Polar Lipids (Alabaster, AL). High purity calcein, 4,4-difluoro-5-methyl-4-bora-3a,4a-diaza-s-indacene-3-dodecanoic acid (Bodipy® 500/510C1, C12 or C1,C12-Bodipy), 5-butyl-4,4-difluoro-4-bora-3a,4a-diaza-s-indacene-3-nonanoic acid (Bodipy® 500/510C4C9 or C4, C9-Bodipy), 4,4-difluoro-5-octyl-4-bora-3a,4a-diaza-s-indacene-3-pentanoic acid (BODIPY® 500/510C8,C5 or C8,C5-Bodipy), and 4,4-difluoro-5-(4-phenyl-1,3-butadienyl)-4-bora-3a,4a-diaza-*s*-indacene-3-undecanoic acid (C_{11}-Bodipy® 581/591) were purchased from Invitrogen (Carlsbad, CA). Potassium phosphate monobasic, potassium phosphate dibasic, Sephadex G-75 column beads, columns and sodium chloride were purchased from Fisher Scientific. F_2G, a byproduct from pilot-scale production of feruloyl soy glycerols, was isolated as previously detailed by Compton and Laszlo (2009). FG was synthesized as previously described by Compton et al. (2012).

2.2. Preparation unilamellar phospholipid Vesicles

Lipid mixtures and vesicles were prepared as described previously (Laszlo et al., 2010, 2011). Mixtures were prepared from appropriate amounts of DOPC in chloroform and FG or F_2G (at 1- or 5-mol% of total lipids) in ethanol added to clean amber vials and then gently mixed. Solvents were removed from the lipids by a stream of argon and overnight vacuum. The dried lipids were rehydrated in the appropriate buffer and mixed periodically over an hour to form multilamellar phospholipid vesicles (MLVs). The MLVs were subjected to freeze–thaw cycles and then extrusion to form unilamellar vesicles (EUVs). Extrusion was accomplished by eleven passes through two 100-nm filters stacked within a LiposoFast hand-held extruder (Avestin, Inc., Ottawa, Canada). EUVs were covered with argon, protected from light and stored at 4 °C until used. Vesicles were an average of 109 ± 5 nm in diameter and a polydispersity of 0.1 as determined using dynamic light scattering via a Nicomp particle sizer (Particle Sizing System, Inc., Santa Barbara, CA).

2.3. Partitioning of FA, FG and F_2 into DOPC vesicles

2.3.1. Partition coefficient determination using derivative spectrophotometry

The partition coefficient (K_P) of FG and F_2G were determined in DOPC EUVs in 10 mM HEPES, pH 7.4, buffer following the method described by Ferreira et al., 2003. Briefly, FG or F_2G (40 μM final concentration) was dried in amber vials under argon and hydrated in buffer. Later, EUVs (preparation described above) at 0 to 1.0 mM were added to the vials, stirred and incubated in an oven at 37 °C for 30 min in the dark. Absorbance spectra were recorded over the range of 250–400 nm using a Shimadzu 1240 UV–vis spectrophotometer. Measurements were done in 1-cm path length quartz cells and replicated at least in triplicate.

It is possible to determine K_P for a molecule partitioning into lipid membranes from absorbance measurements, provided that the molecule's absorbance shifts as the concentration of vesicles changes (Santos et al., 2003). However, vesicles produce a high scattering background signal in the UV range that is not always removable by subtracting a background reference as demonstrated by Rodrigues et al. (2001), where absorbance of the partitioning molecule of interest decreased with increasing vesicle concentration. It was demonstrated that the high scattering background from vesicles in the UV range is minimized by using derivative spectrophotometry (Rodrigues et al., 2001; Santos et al., 2003).

The partition coefficient (K_P) between aqueous solution and vesicles is defined as the ratio of the number of molecules embedded into the vesicular membrane media and the number of molecules within aqueous solution, which can be expressed as follows:

$$K_p = \frac{[F]_M}{[F^T]_W} \tag{1}$$

where F represents the feruloyl moiety concentration either within the lipid membrane (M) or water (W); T represents the total overall starting concentration. Additionally, the partition coefficient can be expressed as a function of molar fraction of molecules embedded within the lipid membrane, θ (Ferreira et al., 2003):

$$K_p = \frac{\theta/V_M}{(1-\theta)/V_W}, \tag{2}$$

where V_M and V_W are phase volumes for lipid membrane and water, respectively. The molar fraction is measureable via absorbance,

$$K_p = \frac{A_W - A_L}{A_W - A_M}, \tag{3}$$

where A is the absorbance for molecules in the aqueous solution without any lipids (w), absorbance of molecules in a vesicle solution (L), and the maximum absorbance when all molecules of interest are embedded within the membrane (M), respectively. Eqs. (2) and (3) can be rewritten to express absorbance of molecules in vesicles (A_L) as follows:

$$A_L = A_W + \frac{(A_M - A_W)K_P[L]V_\phi}{1 + K_P[L]V_\phi}, \tag{4}$$

where V_Φ is lipid molar volume. High scattering of vesicles in the UV range can be eliminated using the derivative of the absorbance. Thus, the partition coefficient K_P can be determined by fitting the absorbance derivative to the Eq. (5) (Ferreira et al., 2003; Lúcio et al., 2009):

$$D_T = D_W + (D_m - D_w)\frac{K_P \times L \times V_\phi}{1 + K_P \times L \times V_\phi}; \tag{5}$$

where D_T,D_w, and D_m were the derivative of the total, lipid and aqueous absorbance for FG or F_2G, respectively. Alternatively, partitioning was determined using the n-octanol/water method based on the description given by Turina et al. (2006). Briefly, an aliquot of FG, or F2G in organic solvent was dried under argon in a vial and equal amounts of nanopure water and *n*-octanol (6 ml each) were added to give a final concentration of 40 μM of FG, or F_2G in a 1/1 *n*-octanol/water *n*-octanol. The mix was stirred and equilibrated overnight in the dark. The solution was centrifuged at 1000 g for 10 min to ensure phase separation. Absorbance was measured for the water (A_w) and *n*-octanol (A_o)