JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

Probing the Interaction of Polyphenols with Lipid Bilayers by Solid-State NMR Spectroscopy

Xueting Yu, Shidong Chu, Ann E. Hagerman, and Gary A. Lorigan*

Department of Chemistry and Biochemistry, Miami University, Oxford, Ohio 45056, United States

ABSTRACT: Polyphenols are bioactive natural products that appear to act against a wide range of pathologies. Mechanisms of activity have not been established, but recent studies have suggested that some polyphenols bind to membranes. This study examined the interaction between lipid bilayers and three structurally diverse polyphenols. It was hypothesized that features of the polyphenols such as polarity, molecular size, molecular geometry, and number and arrangement of phenol hydroxyl groups would determine the tendency to interact with the bilayer. The examined compounds included a mixed polyphenol, (−)-epigallocatechin gallate (EGCg); a proanthocyanidin trimer comprising catechin-(4→8)-catechin-(4→8)-catechin (cat_3); and a hydrolyzable tannin, 1,2,3,4,6-penta-*O*-galloyl-β-D-glucopyranose (PGG). These polyphenols were incorporated at different levels into ^{2}H-labeled 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) multilamellar vesicles (MLVs). ^{31}P and ^{2}H solid-state NMR experiments were performed to determine the dynamics of the headgroup region and the hydrophobic acyl chain region of the lipid bilayer upon addition of polyphenols. The chemical shift anisotropy (CSA) width of the ^{31}P NMR spectra decreased upon addition of polyphenols. Addition of PGG induces a dramatic reduction on the CSA width compared with the control lipid bilayer sample, whereas addition of cat_3 barely reduces the CSA width. The ^{2}H quadupolar splitting of the lipids also decreased upon addition of polyphenols. At the same concentration, PGG substantially reduced the quadrupolar splitting, whereas cat_3 barely reduced it when compared with the control sample. From a calculation of the order parameters of the acyl chain region of the lipid bilayer, it was concluded that the hydrophobic part of the lipid bilayer was perturbed by PGG, whereas cat_3 did not cause large perturbations. The data suggest that the polarity of the polyphenols affects the interaction between tannins and membranes. The interactions may relate to the biological activities of polyphenols.

KEYWORDS: polyphenols, lipid bilayers, polarity, molecular size, molecular geomtry, biological activities, proanthocyanidin, hydrolyzable tannin, MLVs, solid-state NMR

INTRODUCTION

Polymeric polyphenols (tannins) are natural products found in virtually all dicotyledonous higher plants. The hallmark of this diverse group of compounds is their ability to interact strongly with proteins, yielding very stable and often insoluble complexes.[1] Polyphenols are divided into three classes on the basis of their monomer units: proanthocyanidins comprise flavan-3-ols, which may or may not be 3-galloylated, polymerized via C−C bonds; hydrolyzable tannins are gallic acid esters of glucose; and the galloylated catechins are gallate esters of simple flavan-3-ols.[2] Recent interest in polyphenols has been focused on their diverse bioactivities and their potential benefits as phytonutrients or pharmacological agents.[3−5]

Neither the avid protein binding[1] nor the potent antioxidant activities of polyphenols[6] adequately account for all of the bioactivities noted for polyphenols. Some recent studies of the galloylated catechins have suggested that this class of polyphenols may interact with membranes[7−17] and that those interactions may be the basis for some polyphenol bioactivities. For example, it has been suggested that the antibacterial effect of catechin and its derivatives may be a consequence of increased membrane fluidity induced by the polyphenols.[13] Catechin and its derivatives may adsorb to membrane surfaces and insert into the lipid bilayer, causing fatal perturbations to membrane structure.[13] Proanthocyanidins and hydrolyzable tannins have bioactivity profiles similar to those of the galloyated catechins, but the interactions of these classes of polyphenols with membranes have not been explored in detail. One goal of our study was to evaluate the interactions between a model membrane system and a representative compound from each of the three main classes of polyphenols.

Interactions with the lipid bilayer are well established for the galloylated catechins such as EGCg, with variation related to the structural properties of the polyphenol.[7,14] Sirk and co-workers proposed that polyphenols form hydrogen bonds with membranes, with phenolic hydroxyl groups serving as the hydrogen bond donors and oxygen atoms on the phospholipid as the hydrogen bond acceptors.[8] They concluded that the strength of the interaction depends on the number of hydrogen bonds formed and, thus, on both the degree of hydroxylation and the stereochemical features of polyphenols. For instance, EGCg and (−)-gallocatechin-3-gallate (GCg) are epimers that are cis (epigallocatechin) and trans (gallocatechin) at C2 and C3. EGCg interacts with the lipid more strongly than GCg, perhaps because the parallel arrangement of rings B and D in EGCg promotes hydrogen bond formation.

Solid-state NMR has been used to examine the interaction between [4-^{2}H]EGCg and multilamellar vesicles (MLVs).[14,15] This work established that EGCg alters the dynamics of the lipid bilayer. The data suggested that the B and D rings of EGCg insert into the bilayer and contact the inner hydrophobic region. To interact with membrane, the polyphenol must be favorably oriented with the hydrophobic domains (rings B and D) in direct contact with the

Received: January 14, 2011
Revised: March 28, 2011
Accepted: May 16, 2011
Published: May 16, 2011

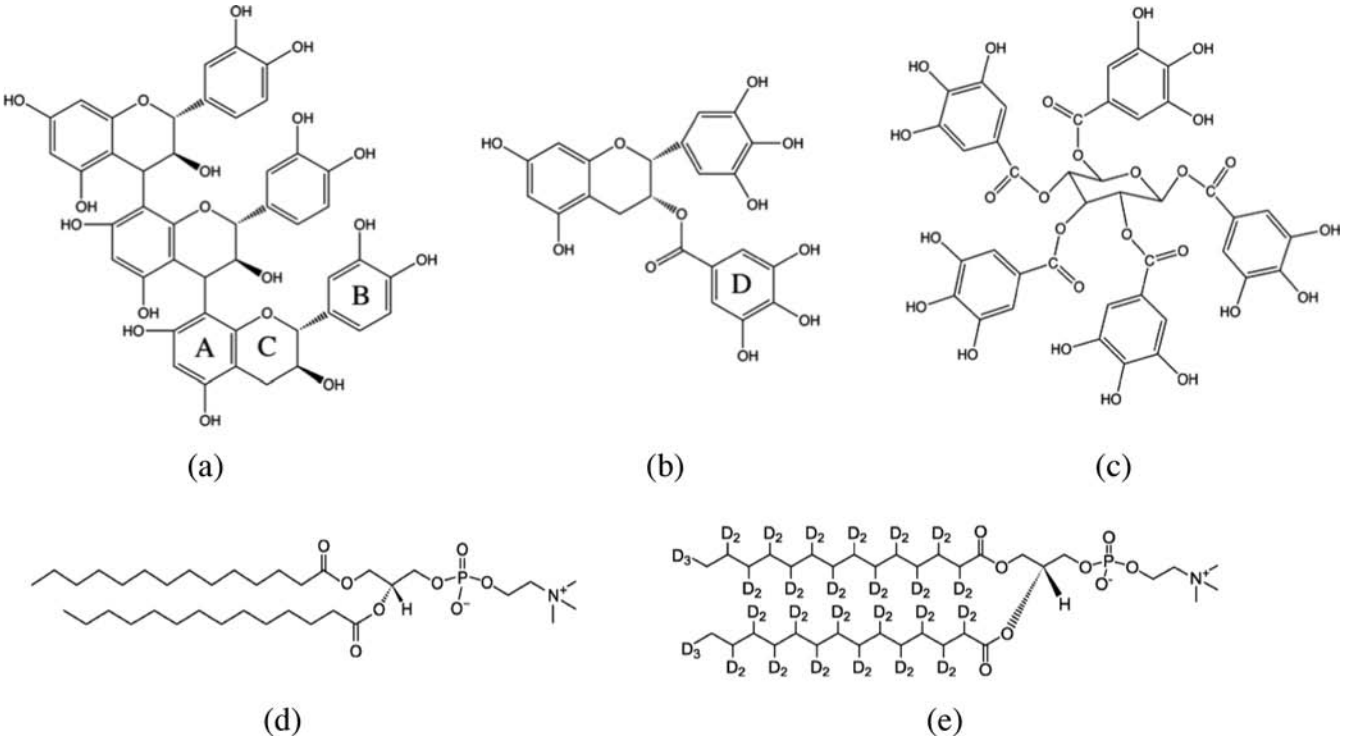

Figure 1. Structural formulas of (a) cat_3, (b) EGCg, (c) PGG, (d) DMPC, and (e) DMPC-d_{54}.

membrane. This model of interaction does not invoke hydrogen bonding between the polyphenol and the membrane. These two contrasting models for polyphenol−membrane interactions have not been resolved to date. One goal of our study was to compare the interactions between membranes and several polyphenols that have very different polarities to further evaluate the relative importance of hydrogen bonding and hydrophobic interactions.

We have examined representatives of the three classes of polyphenols: a proanthocyanidin trimer comprising catechin-(4→8)-catechin-(4→8)-catechin (cat_3); a hydrolyzable tannin, 1,2,3,4,6-penta-*O*-galloyl-β-D-glucopyranose (PGG); and a galloylated catechin, (−)-epigallocatechin gallate (EGCg) (Figure 1). All three compounds are highly hydroxylated (cat_3, 12 phenolic hydroxyls; PGG, 15; EGCg, 8), but their polarities are quite different. Cat_3 is quite polar, with an octanol−water partition coefficient (K_{ow}) of 0.12, EGCg somewhat less polar (K_{ow} = 12), and PGG extremely hydrophobic (K_{ow} = 100).[1,18] Studying the interactions of these three representative polyphenols with membranes should provide new mechanistic insights into possible modes of bioactivity for polyphenols.

We chose to use solid-state NMR spectroscopy to study the structural and dynamic properties of molecules incorporated into membrane lipid bilayers.[19] MLVs, which comprise many stacked layers of lipid bilayer, are a useful model system for examining the interactions between the membrane and membrane proteins or other molecules with NMR.[20,21] ^{31}P NMR is commonly used to study the headgroup motion and structure of the membrane lipid bilayer.[19,22] ^{2}H NMR spectroscopy provides information on the dynamics of the hydrophobic regions of the membrane, by incorporating phospholipids labeled with ^{2}H on the acyl chain.[23−28]

In this study, model compounds representing each of the three kinds of polyphenols were investigated with the well-defined 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) MLV membrane model.[18,19] ^{31}P and ^{2}H solid-state NMR spectroscopic techniques were employed to reveal the dynamics of the lipid bilayer upon addition of the polyphenols to ^{2}H-labeled MLVs. We hypothesized that the interaction between the polyphenols and the lipid bilayers would be governed by a combination of structural features including polarity, molecular size, molecular geometry, and number and arrangement of phenolic hydroxyl groups.

MATERIALS AND METHODS

The proanthocyanidin trimer cat_3 (Figure 1a) was a generous gift from H. H. Kolodziej,[29] and the galloylated catechin EGCg (Figure 1b) was a generous gift from Douglas Balentine (Lipton Tea, Englewood Cliffs, NJ). The hydrolyzable tannin PGG (Figure 1c) was prepared by methanolysis from commercial tannic acid.[30] The polyphenols were kept in a −20 °C freezer before use. The lipids DMPC (Figure 1d) and 1,2-dimyristoyl(d_{54})-*sn*-glycero-3-phosphocholine (DMPC-d_{54}) (Figure 1e) were purchased from Avanti Polar Lipids (Alabaster, AL) and were dissolved in chloroform at 10 and 50 mg/mL, respectively. *N*-[2-Hydroxyethyl]piperazine-*N*′-2-ethanesulfonic acid (HEPES) was obtained from Sigma-Aldrich (St. Louis, MO) and deuterium-depleted water from Cambridge Isotope Laboratories, Inc. (Andover, MA); reagent grade sodium chloride and methanol were purchased from Fisher Scientific (Pittsburgh, PA).

NMR Sample Preparation. Polyphenols were incorporated into the lipid bilayers according to the method of Rigby and co-workers.[23] ^{2}H-Labeled MLVs were prepared by mixing DMPC and DMPC-d_{54} at a molar ratio of 9:1, using 0.045 mmol of DMPC and 0.005 mmol of DMPC-d_{54}. Polyphenols were dissolved in a minimal volume of methanol and then mixed with the lipids to reach the desired concentration (2.5, 5, or 10 mol % relative to the lipids). The lipids and the dissolved polyphenols were placed into a 12 mm × 75 mm test tube, the solvent was removed with a steady stream of N_2 gas, and any residual solvent was removed by transferring the test tube into a vacuum desiccator and drying overnight. The lipid and polyphenol mixture was rehydrated by the addition of 47.5 μL of pH 6.0 HEPES buffer (30 mM HEPES, 20 mM NaCl in

deuterium-depleted water), dissolved by four or five freeze−thaw cycles to achieve homogeneity of MLV,[21] and then transferred into a 4 mm solid-state NMR rotor.

Solid-State NMR Spectroscopy. A Bruker Avance 500-MHz solid-state NMR spectrometer operating at 202.4 MHz with a Bruker 4 mm double-resonance CP-MAS probe was used to record the ^{31}P NMR spectra. A total of 1024 transients were averaged for each spectrum, and line broadening was set to 200 Hz. The spectral width was set to 150 ppm. ^{31}P NMR spectra were collected with the spin echo pulse sequence (90°−τ_1−180°−τ_2−acquire) with ^{1}H decoupling. The 90° pulse was 4.2 μs, and the first and second echo delay, τ_1 and τ_2, were 20 and 14 μs, respectively. The recycle delay was 5 s.

^{2}H NMR spectra were recorded at 76.77 MHz with the same spectrometer and probe. The quadrupolar echo pulse sequence was employed using quadrature detection with complete phase cycling of the pulse pairs. The 90° pulse length was 3 μs, the interpulse delay was 40 μs, and the recycle delay was 0.3 s. The spectral width was 100 kHz, and line broadening was set to 200 Hz. A total of 40960 transients were averaged for each spectrum. All of the samples were equilibrated at 35 °C for at least 10 min before signal acquisition.

NMR Data Analysis. DMFIT software was used to simulate the ^{31}P NMR spectra.[31] The spectra can be fit to a sum of lines that correspond to a minimum number of species that contribute to the ^{31}P NMR line shape. The chemical shift anisotropy (CSA) width is obtained from the simulation.

^{2}H NMR spectra were deconvoluted (dePaked) according to the algorithm of McCabe and Wassall[32,33] so that the lipid bilayer normal was perpendicular to the direction of the static magnetic field. The order parameters can be calculated from the quadrupolar splitting, which can be measured from the dePaked spectra according to the following expression:[28,34]

$$\Delta\nu^i_Q = 3/4(e^2qQ/h)S^i_{CD}$$

$\Delta\nu^i_Q$ is the quadrupolar splitting of a deuteron attached to the ith carbon. e^2qQ/h is the quadrupolar splitting constant (168 kHz for the deuterons in C−^{2}H bonds). S^i_{CD} is the chain order parameter of a deuteron attached to the ith carbon of the acyl chain of DMPC. The ^{2}H nuclei attached to the terminal methyl carbons were designated carbon 14 (carboxylate carbon is carbon 1). The remaining ^{2}H nuclei were assigned in decreasing order along the phospholipid acyl chain. The quadrupolar splittings of the dePaked ^{2}H spectra reveal the order parameters of the C−D methylene groups and the terminal methyl groups of the acyl chain. The quadrupolar splitting of the CD_3 methyl groups at the end of the acyl chains is the smallest and closest to 0 kHz because they rotate at the fastest frequency. The next smallest quadrupolar splitting was assigned to the ^{2}H attached to C-13 and so forth along the acyl chain.[27] The order parameters calculated for the CD_3 quadrupolar splitting were multiplied by 3 according to the literature.[18,21]

RESULTS

^{31}P NMR Spectroscopy of Polyphenols in Phospholipid Bilayers. ^{31}P solid-state NMR spectra were collected to study the interaction between the headgroup region of the lipid bilayers and cat_3, EGCg, or PGG. The polyphenols were studied at different concentrations between 2.5 and 10 mol % with respect to the lipid. The static ^{31}P NMR spectra of the three polyphenols incorporated into DMPC/DMPC-d_{54} MLVs were recorded at 35 °C, which is near human physiological temperature and is higher than the phase transition (L_α) temperature of DMPC (23 °C) (Figures 2 and 3). Thus, the MLVs were examined in the liquid-crystalline phase.

Cat_3 has very little effect on the lipid bilayer mobility. When incorporated in the MLVs at either 5 or 10 mol %, cat_3 yielded ^{31}P spectra with axially symmetric powder pattern lineshapes very similar to those of the control (Figure 2). At 10 mol % cat_3, the ^{31}P CSA width was slightly smaller than the CSA width of the control sample or the 5 mol % cat_3 sample (Table 1). The spectra indicate that the lipid bilayer remains in the L_α phase with respect to DMPC/DMPC-d_{54} with the addition of up to 10 mol % of cat_3.

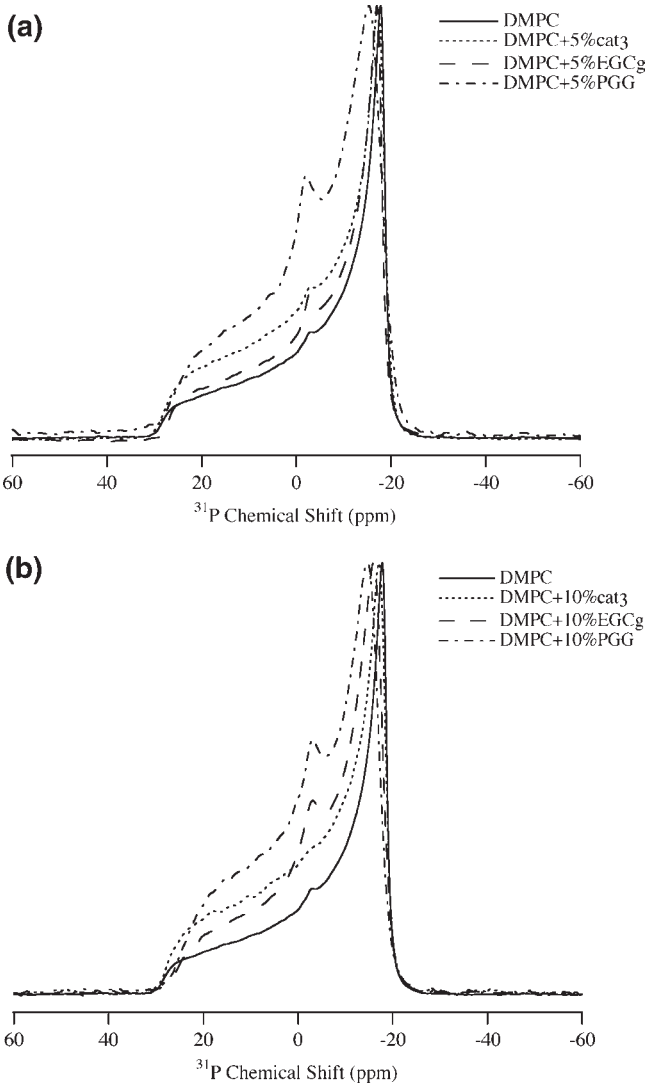

Figure 2. ^{1}H-Decoupled ^{31}P NMR powder spectra of MLV samples of DMPC/DMPC-d_{54} containing various amounts of the polyphenols: (a) bilayers with 5 mol % of three polyphenols (cat_3, EGCg, and PGG, respectively); (b) bilayers with 10 mol % of three polyphenols (cat_3, EGCg, and PGG, respectively). Spectra were collected using a Hahn-echo pulse sequence under static condition at 35 °C.

EGCg increases the mobility of the lipid bilayer in a concentration-dependent fashion. With the addition of EGCg, the CSA width of the ^{31}P NMR spectra decreases relative to the control sample, with a 3.4 ppm decrease in CSA width for samples with 10 mol % EGCg (Table 1). Furthermore, an isotropic peak appears in the spectra upon the addition of EGCg, with more pronounced changes at the higher level of polyphenol (Figure 2).

PGG affects bilayer structure more significantly than EGCg at the same concentration (Figure 2). An isotropic peak at 0 ppm is observed at the lowest level of PGG tested (2.5 mol %)

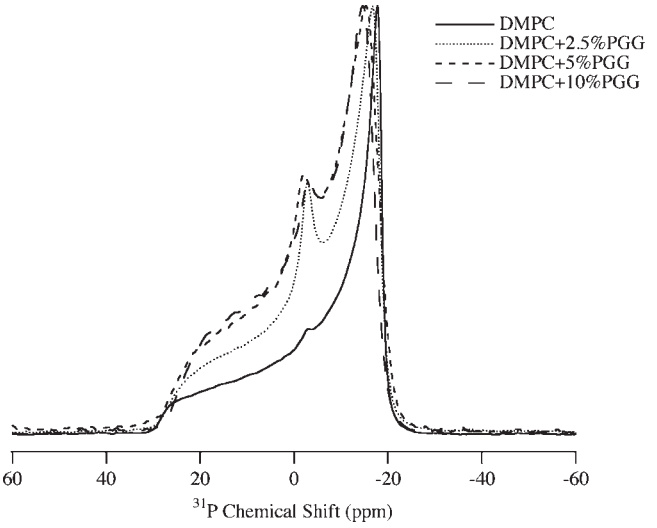

Figure 3. ^{1}H-Decoupled ^{31}P NMR powder spectra of MLV samples of DMPC/DMPC-d_{54} and bilayers with 2.5, 5, and 10 mol % of the polyphenol PGG. Spectra were collected using a Hahn-echo pulse sequence under static condition at 35 °C.

Table 1. Effects of Three Polyphenols (cat_3, EGCg, and PGG) at Concentrations of 5 and 10 mol % on the ^{31}P CSA Width of DMPC/DMPC-d_{54} Bilayers Using ^{31}P Solid-State NMR Experiments under Static Condition at 35 °C

sample DMPC/DMPC-d_{54}	^{31}P CSA (ppm) (±0.2)
control	47.0
5% cat_3	46.5
10% cat_3	45.8
5% EGCg	45.1
10% EGCg	43.6
2.5% PGG	44.3
5% PGG	43.1
10% PGG	41.3

(Figure 3). The CSA width is decreased by PGG, with 5 mol % PGG changing the CSA as much as 10 mol % EGCg (Table 1). As little as 2.5 mol % of PGG reduces the CSA width of the spectrum by about 2.7 ppm when compared with the control lipid sample.

^{2}H NMR Spectroscopy of Polyphenols in Phospholipid Bilayers. Incoporating DMPC-d_{54} (deuterated acyl chain) in the lipid bilayer reveals details of the order and dynamics of the acyl chains. We examined the effect of 5 and 10 mol % polyphenols on ^{2}H NMR spectra of DMPC/DMPC-d_{54} lipids (Figure 4). The ^{2}H quadrupolar splittings marginally decrease upon addition of polyphenols when compared to the control spectrum consisting of pure DMPC/DMPC-d_{54}. The reduction in the quadrupolar splittings indicates that the acyl chains of the lipid are somewhat disordered by the addition of the polyphenols. At a given concentration, PGG reduced the quadrupolar splitting more than EGCg, whereas cat_3 barely changed the splitting. For each type of polyphenol, the reduction in quadrupolar splitting is more noticeable at a higher concentration (Figure 4). Isotropic components show up at around 0 ppm with the addition of PGG at all three concentrations (2.5, 5, and 10 mol %). Both 5 and 10 mol % EGCg also induce isotropic components in ^{2}H NMR spectra. This is

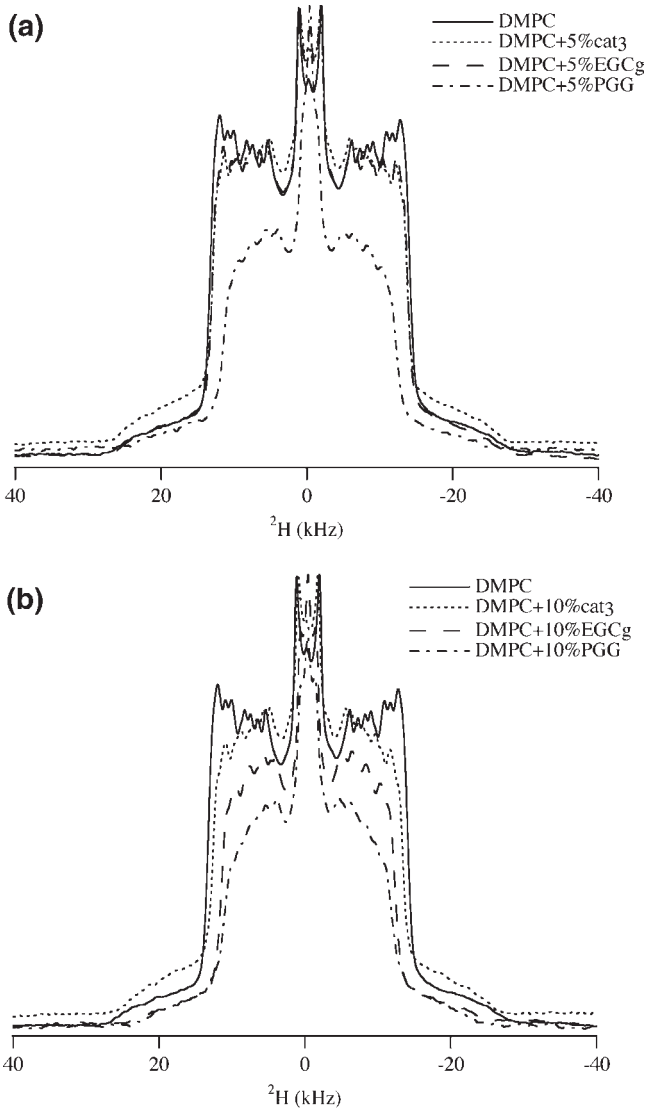

Figure 4. ^{2}H NMR spectra of MLV samples of DMPC/DMPC-d_{54} containing various amounts of the three polyphenols: (a) lipid bilayers with 5 mol % of three polyphenols (cat_3, EGCg, and PGG); (b) lipid bilayers with 10 mol % of three polyphenols (cat_3, EGCg, and PGG). Spectra were collected using a quadrupolar echo pulse sequence under static condition at 35 °C.

consistent with the presence of an isotropic component in ^{31}P NMR spectra of sample containing EGCg or PGG.

All of the ^{2}H NMR spectra were deconvoluted (dePaked) to measure the dynamic changes in the lipids upon addition of polyphenols. The corresponding segmental C−D bond order parameters (S_{CD}) were calculated from the dePaked powder spectra and were plotted as a function of carbon number for each concentration of polyphenol (Figure 5). For control samples, the order parameter of the methylene near the headgroup is the largest, whereas the value decreases along the acyl chain and is smallest for the terminal methyl group (carbon 14) (Figure 6). Compared to the control, addition of polyphenol yields a similar pattern, but the order parameters of the methylene groups are not decreased as much as the terminal methyl group is approached. For each polyphenol, a higher concentration decreased all of the order parameters more than a lower concentration. For the same

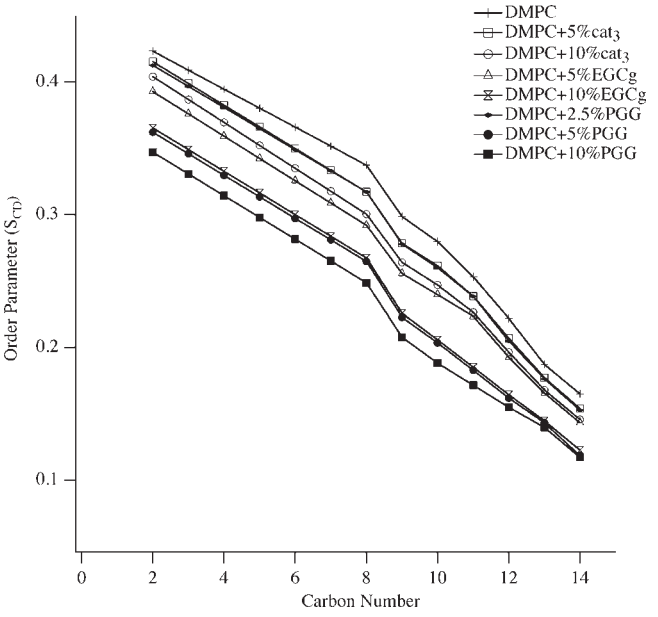

Figure 5. Smoothed acyl chain (DMPC-d_{54}) order parameter (S_{CD}) calculated from the dePaked spectra of DMPC/DMPC-d_{54} and bilayers with three polyphenols (cat_3, EGCg, and PGG) at concentrations of 5 and 10 mol %.

concentration of polyphenolic, the order parameters were reduced the most by PGG and the least by cat_3.

DISCUSSION

Polyphenols have been implicated as agents that provide protection from many chronic diseases including cardiovascular disease, cancer, diabetes, and Alzheimer's disease.[3−5] Well-known activities of polyphenols that might be related to their beneficial bioactivities include their ability to bind protein[1] and their potent antioxidant activity.[6] The ability of polyphenols to interact with the lipid bilayer is much more poorly understood, with mechanistic studies limited to those on the galloylated catechins such as EGCg.[7−17] Interactions with the lipid bilayer are of interest in part because disruption or binding to membranes may mediate specific bioactivities[13] and also because solubility in lipids is a critical determinant of bioavailability. Here, we use a combination of ^{31}P and ^{2}H solid-state NMR spectroscopic techniques to study the dynamics of the lipid bilayer upon addition of representative compounds from the three classes of polyphenols: cat_3, EGCg, and PGG. The proanthocyanidin cat_3 is a very polar polyphenol (K_{ow} = 0.12) with a molecular weight of 864.[1,35] The slightly larger hydrolyzable tannin PGG is very nonpolar (K_{ow} = 100, molecular weight 940).[1,35] The mixed polyphenol EGCg has intermediate polarity (K_{ow} = 12) and is the smallest of the three compounds, with a molecular weight of 458.[1,35] Differences in polarity reflect differences in the three-dimensional structures of the compounds, with cat_3 forming a mixture of extended conformers in solution[36] and PGG forming a hydrophobic sphere.[37]

The reduction in quadrupolar splittings in the ^{2}H NMR spectra upon the addition of PGG to the membrane indicates that PGG significantly disorders the acyl chains of the lipid bilayers. Smaller changes in the quadrupolar splittings obtained with EGCg and cat_3 indicated that these compounds interact more weakly with the acyl chains. Our data indicate that the polarity of the polyphenol is inversely related to the strength of the interaction with the hydrophobic region of DMPC/DMPC-d_{54} lipid bilayer, suggesting

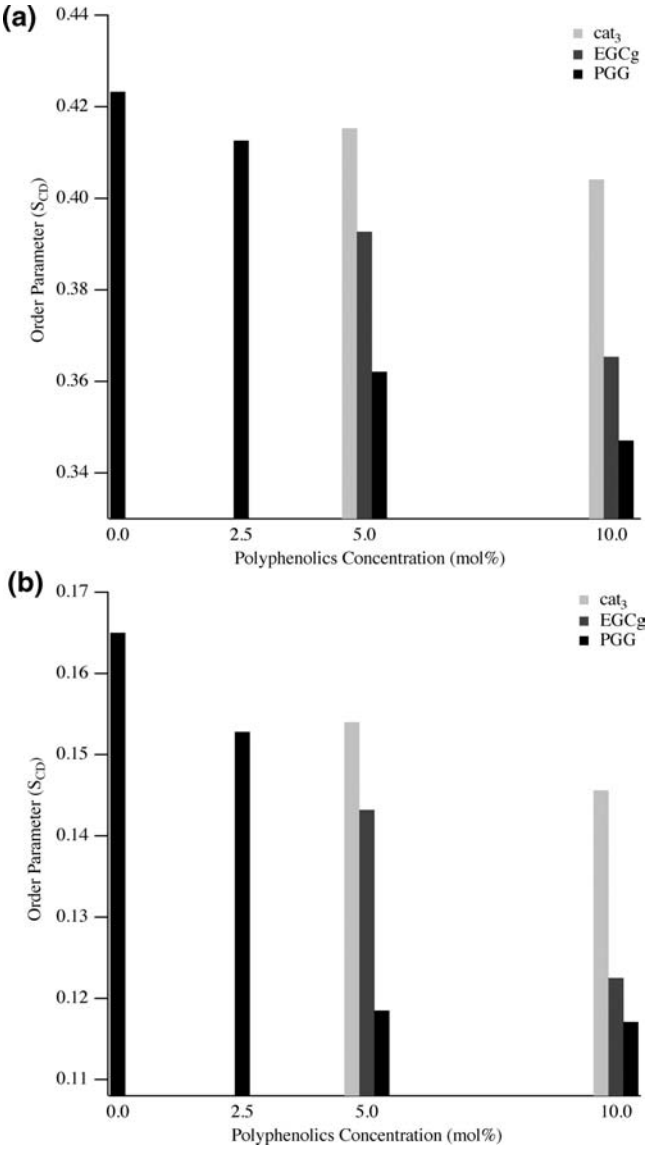

Figure 6. Order parameters of DMPC-d_{54} carbons 2 (a) and 14 (b) upon addition of the three polyphenols (cat_3, EGCg, and PGG) at different concentrations [2.5 mol % (for PGG only), 5 mol %, and 10 mol %].

an important role for hydrophobic forces in polyphenol−membrane interactions.

For DMPC/DMPC-d_{54} bilayers, the order parameters (S_{CD}) decrease as the distance between the C−D bond and the glycerol backbone increases, because there is more motion near the end of the acyl chain than in the headgroup region. For any given methylene group in the acyl chain, the order parameter changes with added polyphenols as follows: no polyphenol > 5 mol % cat_3 > 2.5 mol % PGG > 10 mol % cat_3 > 5 mol % EGCg > 10 mol % EGCg > 5 mol % PGG > 10 mol % PGG. Lower S_{CD} values can be interpreted as increased fluidity of the acyl chain and the increased motion of the vesicles, presumably induced by interactions of polyphenols with the membrane. The order parameters that we obtained for three model polyphenols confirm that strength of interaction between the polyphenol and a bilayer is inversely related to the polarity of the polyphenol.

The ^{31}P NMR spectra are most strongly affected by PGG, whereas cat_3 has little effect. The dramatically reduced CSA width in the ^{31}P NMR spectra for MLVs containing PGG can be attributed to increased fluidity of the membrane surface or decreased size of the vesicles[19] in the presence of this polyphenol. The additional isotropic component that is observed in both ^{31}P and ^{2}H NMR spectra upon addition of EGCg and PGG at all concentrations provides further evidence for the presence of smaller vesicles.[19] The isotropic peak induced by the 5 and 10 mol % of EGCg and PGG suggests that those polyphenols that are sufficiently nonpolar to interact with the bilayer acyl chains may also fragment bigger vesicles into smaller vesicles. The spectral changes noted with addition of polyphenols indicate that these compounds do not aggregate the membrane vesicles, but increase the fluidity or decrease the size of the vesicles.

At the same concentration of polyphenols, the very nonpolar PGG increases the headgroup motion and disorders the acyl chain of the membrane lipid bilayer most significantly when compared with cat_3 and EGCg. The most polar cat_3 trimer disorders the lipid bilayer very little even at 10 mol %. The extent of interaction between the polyphenols and the membrane may depend partly on the polyphenol polarity. Because PGG is the most nonpolar polyphenol, it is expected to interact with the nonpolar acyl chain region deep in the center of the lipid bilayer and to affect the membrane more than the other two polyphenols. The significant decrease of the order parameter of the acyl chain according to the ^{2}H solid-state NMR spectra reflects this interaction (Figure 5). Conversely, the very polar cat_3 trimer has a small effect on order parameters consistent with low interaction with the nonpolar acyl chains. The ^{31}P NMR spectra also confirm the model of hydrophobic interactions. The large perturbation to the membrane headgroup by PGG suggests interaction with the lipid acyl chains that disrupts the membrane overall. The very polar cat_3 does not disturb the head groups, suggesting it is adsorbed on the membrane surface without disturbing it.

The naturally occurring polyphenol tannic acid (TA) has a behavior similar to that of PGG.[38,39] TA is a mixture of galloyl glucoses varying from monogalloyl glucose to dodecagalloyl glucose, with a variable composition depending on source.[40] TA may contain PGG, but it also contains other galloyl glucoses. A ^{2}H powder pattern solid-state NMR study on TA suggested it moderately reduced the order parameter for the acyl chain near the headgroup and much more dramatically affected the terminal region.[39] It was suggested that the first half of the acyl chain tilts cooperatively upon TA insertion, whereas the more mobile terminal region undergoes gauche conformational change, which induces the significant reduction in the order parameter.[38,39] TA induces a moderate reduction of S_{CD} on the membrane surface and a dramatic reduction of S_{CD} in the membrane hydrophobic core. At a similar concentration, PGG induces a larger S_{CD} reduction throughout the lipid acyl chain when compared with TA. The isotropic component that appeared with PGG addition was not observed with TA addition. However, the effect of TA on the S_{CD} of the membrane surface and the acyl chain terminal group is more obvious than that of PGG. Although PGG and TA are structurally similar, it is not surprising that they have different tendencies to interact with membrane. TA is a heterogeneous mixture, so although some components of TA are similar to those of PGG and enter the hydrophobic part of bilayer, other components may only be adsorbed on the membrane surface.[39] PGG is a small, very hydrophobic molecule that is able to interact with both the membrane surface and the hydrophobic core. It has been suggested that at low concentrations TA increases outer membrane surface area and decreases membrane thickness but that at higher concentrations the inner membrane cannot expand or the lipid and TA cannot exchange quickly enough, so the bilayer breaks into smaller vesicles.[39] The isotropic component we noted in both ^{31}P and ^{2}H spectra of the PGG incorporated into the lipid bilayer suggests a similar mechanism for PGG.

Previous studies have indicated that the B ring and the galloyl moiety of EGCg tend to interact with the membrane surface.[9,14,15] Our work extends those previous studies by using MLVs instead of the smaller bicelles or unilamellar vesicles used earlier and by using lower polyphenol concentrations to prevent aggregation of the lipid. Under our conditions EGCg interacted weakly with the acyl groups in the membrane and had intermediate effects on the polar head groups. This is consistent with earlier studies[9,14,15] and, like those studies, suggests that EGCg bioavailability might be limited by its limited ability to penetrate the membrane.

In conclusion, structural features can affect the level of interaction between polyphenols and lipid bilayers. Compounds representing the three major types of naturally occurring polyphenols, cat_3, EGCg, and PGG, were investigated by solid-state NMR methods. The most nonpolar compound, PGG, perturbed the membrane most, supporting hydrophobic models for polyphenol−membrane interactions rather than hydrogen-bonding models. Our data demonstrating limited interactions between cat_3 and membranes suggest that the limited bioavailability of proanthocyanidins[41] may be in part due to their membrane insolubility. The bioavailability of PGG has not been established in vivo,[4] but transport studies in Caco cells suggest that PGG uptake may involve a carrier protein.[42] Our study with MLVs suggests high bioavailability for PGG and related nonpolar tannins on the basis of their strong tendency to interact with membranes. Furthermore, our data suggest that attempts to increase natural product bioavailability by microencapsulation in membrane vesicles ("phytosomes")[43] may have varying success depending largely on the hydrophobicity of the polyphenol and its tendency to interact with bilayers. Additional studies to examine the effect of proteins such as the salivary tannin-binding proteins[42] on the interaction between polyphenols and membrane bilayers may provide further insights into the bioavailability and bioactivities of this widespread class of compounds.

AUTHOR INFORMATION

Corresponding Author

*Phone: (513) 529-3338. E-mail: garylorigan@muohio.edu.

Funding Sources

This work was supported by NIGMS/NIH Grant GM60259-01 and the NSF (CHE-0645709 and MRI-0722403).

REFERENCES

(1) Hagerman, A. E.; Rice, M. E.; Ritchard, N. T. Mechanisms of protein precipitation for two tannins, pentagalloyl glucose and epicatechin(16) (4→8) catechin (procyanidin). *J. Agric. Food Chem.* **1998**, *46*, 2590–2595.

(2) Yoshida, T.; Hatano, T.; Ito, H. High molecular weight plant polyphenols (tannins): prospective functions. In *Chemical Ecology and Phytochemistry of Forest Ecosystems*; Romeo, J. T., Ed.; Elsevier: San Diego, CA, 2005; pp 163−190.

(3) Zaveri, N. T. Green tea and its polyphenolic catechins: medicinal uses in cancer and noncancer applications. *Life Sci.* **2006**, *78*, 2073–2080.

(4) Zhang, J. H.; Li, L.; Kim, S. H.; Hagerman, A. E.; Lu, J. X. Anti-cancer, anti-diabetic and other pharmacologic and biological activities of penta-galloyl-glucose. *Pharm. Res.* **2009**, *26*, 2066–2080.

(5) Aron, P. M.; Kennedy, J. A. Flavan-3-ols: nature, occurrence and biological activity. *Mol. Nutr. Food Res.* **2008**, *52*, 79–104.

(6) Hagerman, A. E.; Riedl, K. M.; Jones, G. A.; Sovik, K. N.; Ritchard, N. T.; Hartzfeld, P. W.; Riechel, T. L. High molecular weight plant polyphenolics (tannins) as biological antioxidants. *J. Agric. Food Chem.* **1998**, *46*, 1887–1892.

(7) Sun, Y.; Hung, W. C.; Chen, F. Y.; Lee, C. C.; Huang, H. W. Interaction of tea catechin (−)-epigallocatechin gallate with lipid bilayers. *Biophys. J.* **2009**, *96*, 1026–1035.

(8) Sirk, T. W.; Brown, E. F.; Sum, A. K.; Friedman, M. Molecular dynamics study on the biophysical interactions of seven green tea catechins with lipid bilayers of cell membranes. *J. Agric. Food. Chem.* **2008**, *56*, 7750–7758.

(9) Uekusa, Y.; Kamihira, M.; Nakayama, T. Dynamic behavior of tea catechins interacting with lipid membranes as determined by NMR spectroscopy. *J. Agric. Food Chem.* **2007**, *55*, 9986–9992.

(10) Caturla, N.; Vera-Samper, E.; Villalain, J.; Mateo, C. R.; Micol, V. The relationship between the antioxidant and the antibacterial properties of galloylated catechins and the structure of phospholipid model membranes. *Free Radical Biol. Med.* **2003**, *34*, 648–662.

(11) Tsuchiya, H. Effects of green tea catechins on membrane fluidity. *Pharmacology* **1999**, *59*, 34–44.

(12) Hashimoto, T.; Kumazawa, S.; Nanjo, F.; Hara, Y.; Nakayama, T. Interaction of tea catechins with lipid bilayers investigated with liposome systems. *Biosci., Biotechnol., Biochem.* **1999**, *63*, 2252–2255.

(13) Kajiya, K.; Hojo, H.; Suzuki, M.; Nanjo, F.; Kumazawa, S.; Nakayama, T. Relationship between antibacterial activity of (+)-catechin derivatives and their interaction with a model membrane. *J. Agric. Food Chem.* **2004**, *52*, 1514–1519.

(14) Kajiya, K.; Kumazawa, S.; Naito, A.; Nakayama, T. Solid-state NMR analysis of the orientation and dynamics of epigallocatechin gallate, a green tea polyphenol, incorporated into lipid bilayers. *Magn. Reson. Chem.* **2008**, *46*, 174–177.

(15) Kumazawa, S.; Kajiya, K.; Naito, A.; Saito, H.; Tuzi, S.; Tanio, M.; Suzuki, M.; Nanjo, F.; Suzuki, E.; Nakayama, T. Direct evidence of interaction of a green tea polyphenol, epigallocatechin gallate, with lipid bilayers by solid-state nuclear magnetic resonance. *Biosci., Biotechnol., Biochem.* **2004**, *68*, 1743–1747.

(16) Tamba, Y.; Ohba, S.; Kubota, M.; Yoshioka, H.; Yamazaki, M. Single GUV method reveals interaction of tea catechin (−)-epigallocatechin gallate with lipid membranes. *Biophys. J.* **2007**, *92*, 3178–3194.

(17) Yoshioka, H.; Haga, H.; Kubota, M.; Sakai, Y. Interaction of (+)-catechin with a lipid bilayer studied by the spin probe method. *Biosci., Biotechnol., Biochem.* **2006**, *70*, 395–400.

(18) Dufourc, E. J.; Parish, E. J.; Chitrakorn, S.; Smith, I. C. P. Structural and dynamical details of cholesterol lipid interaction as revealed by deuterium NMR. *Biochemistry* **1984**, *23*, 6062–6071.

(19) Dave, P. C.; Tiburu, E. K.; Damodaran, K.; Lorigan, G. A. Investigating structural changes in the lipid bilayer upon insertion of the transmembrane domain of the membrane-bound protein phospholamban utilizing P-31 and H-2 solid-state NMR spectroscopy. *Biophys. J.* **2004**, *86*, 1564–1573.

(20) Traikia, M.; Langlais, D. B.; Cannarozzi, G. M.; Devaux, P. F. High-resolution spectra of liposomes using MAS NMR. The case of intermediate-size vesicles. *J. Magn. Reson.* **1997**, *125*, 140–144.

(21) Stockton, G. W.; Polnaszek, C. F.; Tulloch, A. P.; Hasan, F.; Smith, I. C. P. Molecular-motion and order in single-bilayer vesicles and multilamellar dispersions of egg lecithin and lecithin−cholesterol mixtures-deuterium nuclear magnetic-resonance study of specifically labeled lipids. *Biochemistry* **1976**, *15*, 954–966.

(22) Chu, S. D.; Hawes, J. W.; Lorigan, G. A. Solid-state NMR spectroscopic studies on the interaction of sorbic acid with phospholipid membranes at different pH levels. *Magn. Reson. Chem.* **2009**, *47*, 651–657.

(23) Rigby, A. C.; Barber, K. R.; Shaw, G. S.; Grant, C. W. M. Transmembrane region of the epidermal growth factor receptor: behavior and interactions via H-2 NMR. *Biochemistry* **1996**, *35*, 12591–12601.

(24) Seelig, A.; Seelig, J. Dynamic structure of fatty acyl chains in a phospholipid bilayer measured by deuterium magnetic-resonance. *Biochemistry* **1974**, *13*, 4839–4845.

(25) Lafleur, M.; Fine, B.; Sternin, E.; Cullis, P. R.; Bloom, M. Smoothed orientational order profile lipid bilayers by H-2 nuclear magnetic resonance. *Biophys. J.* **1989**, *56*, 1037–1041.

(26) Abu-Baker, S.; Lu, J. X.; Chu, S. D.; Brinn, C. C.; Makaroff, C. A.; Lorigan, G. A. Side chain and backbone dynamics of phospholamban in phospholipid bilayers utilizing H-2 and N-15 solid-state NMR spectroscopy. *Biochemistry* **2007**, *46*, 11695–11706.

(27) Huster, D.; Arnold, K.; Gawrisch, K. Influence of docosahexaenoic acid and cholesterol on lateral lipid organization in phospholipid mixtures. *Biochemistry* **1998**, *37*, 17299–17308.

(28) Huster, D.; Yao, Y. L.; Jakes, K.; Hong, M. Conformational changes of colicin Ia channel-forming domain upon membrane binding: a solid-state NMR study. *Biochim. Biophys. Acta: Biomembr.* **2002**, *1561*, 159–170.

(29) Kolodziej, H. Oligomeric flavan-3-ols from medicinal willow bark. *Phytochemistry* **1990**, *29*, 955–960.

(30) Chen, Y.; Hagerman, A. E. Characterization of soluble noncovalent complexes between bovine serum albumin and β-1,2,3,4,6-penta-*O*-galloyl-D-glucopyranose by MALDI-TOF MS. *J. Agric. Food Chem.* **2004**, *52*, 4008–4011.

(31) Massiot, D.; Fayon, F.; Capron, M.; King, I.; Le Calve, S.; Alonso, B.; Durand, J. O.; Bujoli, B.; Gan, Z. H.; Hoatson, G. Modelling one- and two-dimensional solid-state NMR spectra. *Magn. Reson. Chem.* **2002**, *40*, 70–76.

(32) McCabe, M. A.; Wassall, S. R. Fast-Fourier-Transform DePaking. *J. Magn. Reson., Ser. B* **1995**, *106*, 80–82.

(33) McCabe, M. A.; Wassall, S. R. Rapid deconvolution of NMR powder spectra by weighted fast Fourier transformation. *Solid State Nucl. Magn. Reson.* **1997**, *10*, 53–61.

(34) Dave, P. C.; Tiburu, E. K.; Nusair, N. A.; Lorigan, G. A. Calculating order parameter profiles utilizing magnetically aligned phospholipid bilayers for H-2 solid-state NMR studies. *Solid State Nucl. Magn. Reson.* **2003**, *24*, 137–149.

(35) Mueller-Harvey, I.; Mlambo, V.; Sikosana, J. L. N.; Smith, T.; Owen, E.; Brown, R. H. Octanol−water partition coefficients for predicting the effects of tannins in ruminant nutrition. *J. Agric. Food Chem.* **2007**, *55*, 5436–5444.

(36) Tarascou, I.; Ducasse, M. A.; Dufourc, E. J.; Moskau, D.; Fouquet, E.; Laguerre, M.; Pianet, I. Structural and conformational analysis of two native procyanidin trimers. *Magn. Reson. Chem.* **2007**, *45*, 157–166.

(37) Feldman, K. S.; Smith, R. S. Ellagitannin chemistry. First total synthesis of the 2,3- and 4,6-coupled ellagitannin pedunculagin. *J. Org. Chem.* **1996**, *61*, 2606–2612.

(38) Huh, N. W.; Porter, N. A.; McIntosh, T. J.; Simon, S. A. The interaction of polyphenols with bilayers: conditions for increasing bilayer adhesion. *Biophys. J.* **1996**, *71*, 3261–3277.

(39) Simon, S. A.; Disalvo, E. A.; Gawrisch, K.; Borovyagin, V.; Toone, E.; Schiffman, S. S.; Needham, D.; McIntosh, T. J. Increased adhesion between neutral lipid bilayers − interbilayer bridges formed by tannic-acid. *Biophys. J.* **1994**, *66*, 1943–1958.

(40) Romani, A.; Ieri, F.; Turchetti, B.; Mulinacci, N.; Vincieri, F. F.; Buzzini, P. Analysis of condensed and hydrolysable tannins from commercial plant extracts. *J. Pharm. Biomed. Anal.* **2006**, *41*, 415–420.

(41) Shivashankara, K. S.; Acharya, S. N. Bioavailability of dietary polyphenols and cardiovascular diseases. *Open Nutraceuticals J.* **2010**, *3*, 227–241.

(42) Cai, K. H.; Hagerman, A. E.; Minto, R. E.; Bennick, A. Decreased polyphenol transport across cultured intestinal cells by a salivary proline-rich protein. *Biochem. Pharmacol.* **2006**, *71*, 1570–1580.

(43) Kidd, P. M. Bioavailability and activity of phytosome complexes from botanical polyphenols: the silymarin, curcumin, green tea, and grape seed extracts. *Altern. Med. Rev.* **2009**, *14*, 226–246.