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Synthesis, structure, and thermal properties of 1,2-dipalmitoylgalloylglycerol (DPGG), a novel self-adhering lipid

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Abstract

A novel diacyl glycerol-based lipid with a polyphenolic head group has been synthesized and characterized. X-ray diffraction experiments show that this lipid, 1,2-dipalmitoylgalloylglycerol (DPGG), hydrates to form gel phase bilayers at 20°C with extremely narrow interbilayer fluid separations, indicating that apposing DPGG bilayers strongly adhere to each other. Differential scanning calorimetry shows that fully hydrated DPGG exhibits a pretransition exotherm (3.7 kcal/mol) at 52°C and a high enthalpy (11.3 kcal/mol) main endothermic transition at 69°C. These thermal properties are similar to those of galactosylceramides with similar hydrocarbon chain compositions. The adhesive and thermal properties of DPGG are likely due to both intermolecular hydrogen-bonding and hydrophobic interactions between the aromatic rings on the gallic acids.

Keywords: Adhesion; X-ray diffraction; Calorimetry; Polyphenol

1. Introduction

The adhesive properties of lipid bilayers depend on the balance of a number of non-specific attractive and repulsive energies. For most phospholipid bilayers, the major repulsive interactions include electrostatic, hydration, and steric pressures (Parsegian et al., 1979; Rand and Parsegian, 1989; McIntosh and Simon, 1994; Israelachvili and Wennerstrom, 1996), and the major attractive interaction is the van der Waals pressure (Parsegian et al., 1979). However, compared to most membrane lipids, two classes of lipids have unusually large adhesion energies — electrically neutral glycolipids, such as cerebrosides, and the zwitteri-

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onic phospholipid, phosphatidylethanolamine (PE) (Evans and Needham, 1987; McIntosh and Simon, 1996). Both cerebrosides and PEs form hydrogen bonds in the plane of the membrane (Boggs 1987; Pascher et al., 1987), and it has been argued that such H-bond networks may be critical to the adhesive properties of these bilayers (Seddon et al., 1984; Damodaran and Merz, 1994). Related to the strong self-adhesion (and therefore low hydration) of cerebrosides and PEs are their higher melting temperatures (T_m) compared to phosphatidylcholines and phosphatidylglycerols with similar hydrocarbon compositions (Nagle, 1976, 1980; Ruocco and Shipley, 1983). Again, the H-bond characteristics of cerebrosides and PEs could be one factor in their high melting temperatures. In the case of the cerebroside galactosylceramide (GalCer) it has been argued that the higher T_m involves stabilization of the gel phase via intra- and intermolecular H-bonds involving the galactose (Ruocco and Shipley, 1983). In the case of PE, weak and transient H-bonding between the phosphate and ethanolamine groups could raise T_m by inhibiting lateral expansion of the bilayer (Nagle, 1976, 1980).

The adhesion and partial dehydration of neutral phospholipid bilayers can also be promoted by the exogenous addition of tannic acid or other polyphenolic compounds (Simon et al., 1994; Huh et al., 1996). These polyphenols appear to associate with phosphatidylcholine bilayers by hydrophobic interactions arising from the aromatic rings on their gallic acids (Oh et al., 1980), by donating hydrogen bonds to H-bond accepting groups (Haslam, 1974), and by π-dipole interactions between the choline headgroup and the aromatic gallates (Haslam, 1974). By similar types of interactions tannic acid also aggregates or precipitates a variety of proteins and polymers (Goldstein and Swain, 1965; Nash et al., 1966; Haslam, 1974; Oh et al., 1980).

In this paper we synthesize and characterize the new lipid, 1,2-dipalmitoylgalloylglycerol (DPGG), which is a diacyl glycerol-based lipid containing a polyphenolic head group. This study has two primary goals. The first is to develop a lipid molecule that forms bilayers with strong adhesive properties. Such bilayers could potentially be used to tether proteins, membranes, or cells to glass or other substrates for experimental use or as a biocompatible coating for medical implants. The second goal is to compare the structure and thermal properties of DPGG to naturally occurring lipids, such as galactosylceramide and PE, which have large adhesion energies but different hydrogen-bonding patterns. Of particular interest is a comparison of DPGG with GalCer, since the headgroups of both lipids have multiple hydroxy groups, but DPGG lacks the sphingosine backbone that may be important in intramolecular H-bonding in GalCer (Pascher and Sundell, 1977).

2. Materials and methods

2.1. Chemical synthesis

The scheme for the synthesis of 1,2-dipalmitoylgalloylglycerol (**1**) is outlined in Fig. 1. 2,3-Dipalmitoylglycerol (**2**) was obtained from Avanti Polar Lipids (Alabaster, AL). Other chemicals were obtained from Aldrich (St. Louis, MO) and were used as received. Tri-*O*-benzylgalloyl chloride (**3**) was synthesized by benzylation of methyl gallate, followed by hydrolysis, acidification, and treatment of the resulting carboxylic acid with phosphorous pentachloride. Chemical synthesis reactions were run in flame-dried glassware under Argon, and all solvents were distilled. ^1H and ^{13}C NMR spectra were recorded on a Varian INOVA-400 spectrometer. Chemical shifts are reported in ppm using TMS as a reference. FTIR spectra were recorded on a Bomem Michelson Series BM-10 FTIR spectrophotometer and mass spectra were obtained on a JEOL 5X-10Z using fast atom bombardment (FAB). Melting points were obtained on a Hoover apparatus and are uncorrected.

2.1.1. Dipalmitoyl(3,4,5-tri-O-benzylgalloyl)-glycerol (4)

To a solution of 80 mg dipalmitoylglycerol (**2**) (0.1 mmol, 1 eq.) in 25 ml CH_2Cl_2 was added 54 mg tri-*O*-benzylgalloyl chloride (**3**) (0.11 mmol, 1.1 eq.) and 14 mg DMAP (0.11 mmol, 1.1 eq.)

and stirred at room temperature over night. The reaction was diluted with CH_2Cl_2 and washed 2 × 1 M HCl, 2 × 1 M NaOH, 1 × brine, dried over $MgSO_4$, filtered, and evaporated. The residue was chromatographed in 1:1 Et_2O:hexane (Rf = 0.61). Evaporation provided (**4**), mp 56°C, in 72% yield. ^{1}H NMR: ($CDCl_3$, ppm): 7.40 (m, 17H), 5.41 (m, 1H), 5.14 (s, 4H), 5.12 (s, 2H), 4.45 (dd, $J_1 = 4.8$ Hz, $J_2 = 11.6$ Hz, 1H), 4.38 (m, 2H), 4.20 (dd, $J_1 = 6$ Hz, $J_2 = 12$ Hz, 1H), 2.30 (t, 7.6 Hz, 4H), 1.61 (m, 4H), 1.22 (brm, 48H), 0.88 (t, $J = 7.2$ Hz). ^{13}C NMR ($CDCl_3$, ppm): 201.9, 196.2, 152.6, 128.6, 128.2, 128.0, 127.5, 109.2, 75.1, 71.2, 63.0, 62.1, 34.3, 34.0, 31.9, 29.7, 29.5, 29.4, 29.3, 29.1, 24.9, 22.7, 14.1. IR($CHCl_3$ solution): 2926 cm^{-1}, 2853 cm^{-1}, 1725 cm^{-1}. MS (HiRes, Fab +): calculated for $C_{63}H_{90}O_9$ (M +): 990.6585, found: 990.6542. $[\alpha]_D$($CHCl_3$): +0.105.

2.1.2. Dipalmitoylgalloylglycerol (DPGG) (1)

A solution of (**4**) in THF was hydrogenated over 10% Pd/C at 80 psi H_2 overnight, filtered through Celite, and the solvent evaporated. The residue was then dissolved in acetone and treated with decolorizing carbon to provide (**1**), a white solid, mp 88°C, in 75% yield. ^{1}H NMR: ($CDCl_3$, ppm): 7.21 (s, 2H), 6.20 (brs, 3H), 5.44 (m, 1H), 4.39 (m, 3H), 4.26 (m, 1H), 2.34 (t, $J = 7.6$ Hz), 1.61 (m, 4H), 1.25 (brm, 48H), 0.88 (t, $J = 6.8$ Hz). ^{13}C NMR ($CDCl_3$, ppm): 174.0, 173.7, 166.2, 142.6, 120.7, 109.9, 69.1, 63.0, 62.3, 34.3, 34.1, 31.9, 30.3, 29.7, 29.5, 29.4, 29.3, 29.1, 24.9, 22.7, 14.1. IR($CHCl_3$ solution): 3329 cm^{-1} 2926 cm^{-1}, 2853 cm^{-1}, 1725 cm^{-1}. MS (HiRes, Fab$^+$): calculated for $C_{42}H_{73}O_9$ (MH^+) 721.5255, found 721.5255. $[\alpha]_D$($CHCl_3$): +0.040.

2.2. Differential scanning calorimetry

Lipid samples were prepared by weighing DPGG into an aluminum sample pan and adding appropriate amounts of water. The pans were hermetically sealed and cycled repeatedly between 20 and 80°C (which is above the main endothermic lipid transition temperature, Fig. 2). The lipid was then heated and thermograms recorded at a scan rate of 2.5°C/min in a Perkin-Elmer DSC7 calorimeter. The reference cell contained a volume of water equal to the amount in each of the hydrated samples.

2.3. X-ray diffraction

X-ray diffraction patterns of both dry and hydrated DPGG were recorded on Kodak DEF X-ray film. Hydrated DPGG was prepared by addition of excess water and repeated heating and cooling cycles between 20 and 80°C, a tempera-

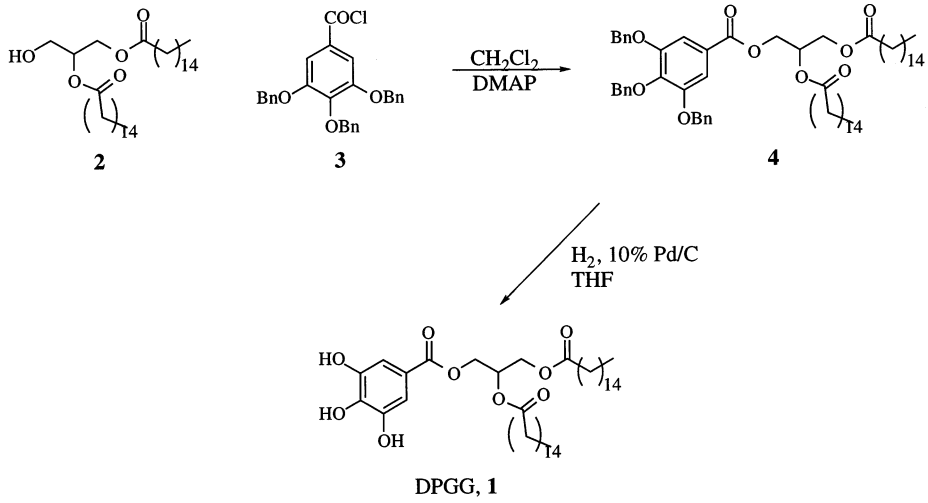

Fig. 1. Scheme for the synthesis of 1,2-dipalmitoylgalloylglycerol (DPGG).

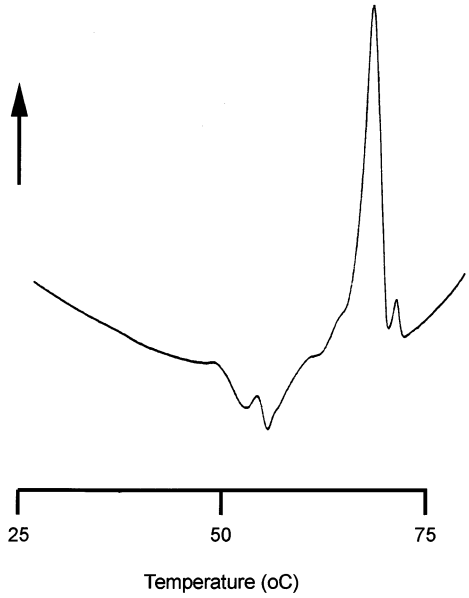

Fig. 2. Differential scanning calorimetry thermogram of fully hydrated DPGG. The arrow points in the direction of an endothermic transition.

ture above the lipid's main transition temperature. The mixture was vortexed extensively. Both the dry powder and hydrated DPGG samples were loaded in quartz glass X-ray capillary tubes and mounted in a point collimation X-ray camera and exposed at 20°C. X-ray films were processed by standard techniques and densitometered with a Joyce-Loebl microdensitometer as described previously (McIntosh and Simon, 1986a,b). After background subtraction, integrated intensities, $I(h)$, were obtained for each order h by measuring the area under each diffraction peak. The structure amplitude $F(h)$ was set equal to $\{h^2 I(h)\}^{1/2}$ (McIntosh and Simon, 1986a). Electron density profiles, $\rho(x)$, on a relative electron density scale were calculated from

$$\rho(x) = (2/d) \sum \exp\{i\phi(h)\} \cdot F(h) \cdot \cos(2\pi x h/d) \quad (1)$$

where x is the distance from the center of the bilayer, d is the lamellar repeat period, $\phi(h)$ is the phase angle for order h, and the sum is over h. The resolution of the profiles was $d/2h_{max} \approx 8$ Å.

3. Results

3.1. Differential scanning calorimetry

Fig. 2 shows a thermogram of DPGG in excess water. DPGG exhibited exothermic pretransition peaks at 52.5 and 55.4°C, a main endothermic transition at 69.3°C with an endothermic shoulder centered at 64°C, and an additional minor endothermic transition at 71.1°C. The total enthalpy of the pretransition peaks (ΔH_{pre}) was -3.7 kcal/mole, whereas the total enthalpy of the main endothermic transition and shoulder (ΔH_m) was 11.3 kcal/mol (Table 1). This thermogram displayed similar features to the one obtained for the fully hydrated galactosylceramide *N*-palmitoylgalactosylsphingosine (NPGS) (Ruocco et al., 1981; Ruocco and Shipley, 1983), which has an exothermic pre-transition at 52°C followed by a main endothermic transition at 82°C (Table 1). However, the thermal transitions of DPGG differed markedly from those of a phosphatidylcholine (DPPC) and a phosphatidylethanolamine (DPPE) with the same hydrocarbon composition as DPGG (Table 1).

Table 1
Thermal properties of DPGG and related lipids[a]

Lipid	T_{pre} (°C)	ΔH_{pre} (kcal/mol)	T_m (°C)	ΔH_m (kcal/mol)
DPGG	52.5, 55.4	−3.7	69.3	11.3
NPGS	52	−0 to −8[b]	82	17.5
DPPC	34.9	1.5	41	8.3
DPPE			64	8.5

[a] NPGS data are from Ruocco et al. (1981), and DPPC and DPPE data are from Marsh (1990).
[b] Depends on the scan rate and thermal history.

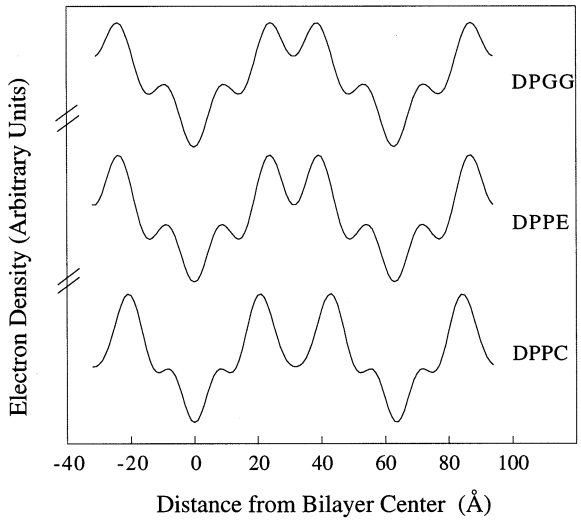

Fig. 3. Electron density profiles for fully hydrated DPGG, DPPE, and DPPC multilayers. Each profile shows two unit cells containing two bilayers and the fluid spacing between apposing bilayers. DPPE and DPPC data are from McIntosh (1980).

3.2. X-ray diffraction

Low angle diffraction patterns of unheated DPGG powder yielded four orders of diffraction corresponding to a lamellar repeat period of 51 Å. There were also seven sharp wide-angle reflections, the two most intense corresponded to 3.93 and 3.67 Å, indicating a crystalline packing of the saturated acyl chains lipids in the plane of the membrane. For comparison, anhydrous *N*-palmitoylgalactosylsphingosine (NPGS) gave a diffraction pattern with a lamellar repeat period of 56 Å, and wide-angle reflections at 4.4 Å (sharp) and 3.8 Å (broad) (Ruocco et al., 1981).

Complete hydration of DPGG, as evidenced by a visible excess water phase, was achieved by addition of excess water and cycling between room temperature and 80°C (which is above T_m). The X-ray pattern for fully hydrated DPGG consisted of four low angle reflections that indexed as orders of a lamellar repeat period 62 Å, and a single sharp wide-angle reflection at 4.09 Å. The sharpness of the single reflection indicates that chains were packed in a hexagonal lattice and not significantly tilted from the bilayer plane, characteristic of an Lβ phase (Tardieu et al., 1973; McIntosh, 1980). This pattern was very similar in both repeat period and intensity distribution to the one obtained at 20°C for dipalmitoylphosphatidylethanolamine (DPPE), which gave a lamellar repeat period of 63 Å and a single sharp wide-angle reflection centered at 4.15 Å (McIntosh, 1980). For comparison, the X-ray patterns of NPGS at full hydration (after being cooled from above its transition temperature) had two lamellar repeat periods of 58 and 55 Å with a sharp wide angle reflection at 4.55 Å and a more diffuse reflection at 4.16 Å (Ruocco et al., 1981).

To obtain more structural information regarding the Lβ gel phase of fully hydrated DPGG, we calculated an electron density profile (top profile in Fig. 3). Due to the similarities in the patterns from DPGG and DPPE, this profile was calculated assuming the same phase angles as previously used for DPPE (McIntosh, 1980). The profile of DPGG (Fig. 3) shows two apposing bilayers with the origin located at the center of the bilayer on the left. For the bilayer on the left the high electron density peaks at ± 25 Å correspond to the relatively high density galloyl head groups, the trough at 0 Å corresponds to the terminal methyl groups of the fatty acid chains, and the medium density regions between the terminal methyl trough and the head group peaks correspond to the fatty acid methylene chains. The medium density region centered at 31 Å corresponds to the center of the fluid space between apposing bilayers.

For comparison, electron density profiles of DPPE and DPPC are also shown in Fig. 3, with each profile containing two unit cells, including two apposing bilayers and the fluid space between each bilayer. The shapes of the profiles were similar for DPGG, DPPE, and DPPC. However, for the three systems the distances between the high density head group peaks varied, both across each bilayer and between adjacent bilayers. The distance between head group peaks across the bilayer hydrocarbon region was 49, 49 and 42 Å for DPGG, DPPE, and DPPC, respectively, whereas the distance between head group peaks from apposing bilayers was 13, 14, and 22 Å for DPGG, DPPE and DPPC, respectively. Another

difference between the profiles from the three lipids was that the electron density of the fluid spacing between apposing bilayers differed. In particular, using the electron density of the hydrocarbon region of the bilayers as a reference, one can see that the electron density of the interbilayer space was greater for DPGG than for DPPC.

4. Discussion

The calorimetry and X-ray diffraction results presented in this paper show that DPGG has similar properties to other lipids, such as cerebrosides and PE, that have large adhesion energies and narrow interbilayer fluid spacings.

4.1. Differential scanning calorimetry

The calorimetry data for DPGG (Fig. 2 and Table 1) were quite similar to those obtained from NPGS, a cerebroside with an amide linked fatty acid with the same acyl chain length as the fatty acids in DPGG. This suggests that the specific H-bonding network found in cerebrosides (in part between the galactose and sphingosine backbone, Pascher and Sundell, 1977) is not necessary to account for all the unusual thermal properties of NPGS and other cerebrosides. For NPGS, the exothermic phase transition (at 52°C) is associated with the hydration of the polar head group occurring at a phase transition between two gel phases, the lower temperature phase having no hydrocarbon chain tilt and the higher temperature phase having tilted chains (Ruocco et al., 1981). Given that the exothermic transition of NPGS and DPGG both occur at the same T_m and have approximately the same enthalpy (Table 1), it is likely that similar phase transitions are present in DPGG. For NPGS the main endothermic transition has $T_m = 82$°C and $\Delta H_m = 17.5$ kcal/mol (Table 1) resulting from a transition between a titled gel phase ($L\beta'$) and liquid–crystalline ($L\alpha$) phase (Ruocco et al., 1981). The main endothermic transition of DPGG has a lower T_m and ΔH_m than NPGS, which likely reflects different packing of the acyl chains for the two lipids and the additional work of breaking up the strong H-bonding between the sphingosine backbone and the galactose moiety in NPGS. Such interactions would not be present in DPGG.

Table 1 also shows that both T_m and ΔH_m were greater for DPGG than for DPPE and DPPC, two glycerol-based phospholipids with the same hydrocarbon chains. Since these three lipids have the same glycerol backbone and acyl chains, it follows that the polar head group is critical. The larger ΔH_m seen for DPGG compared to DPPC or DPPE could derive from several phenomena, including different hydrocarbon chain packing in the gel phase. However, this difference may arise, at least in part, from the additional energy in the gel phase required to break some of the attractive interactions between neighboring gallates.

4.2. X-ray diffraction

The X-ray data clearly show that there are large structural differences between dry and fully hydrated DPGG. Hydration of the lipid converted a crystalline hydrocarbon chain packing into a hexagonal packing, typical for hydrated gel phase phospholipid bilayers with untilted hydrocarbon chains (Tardieu et al., 1973; McIntosh, 1980). In addition, the lamellar repeat period increased when the lipid was hydrated, most likely due to a decrease in chain tilt and possibly a change in hydration and conformation of the polar head group.

Although the electron density profile in the fully hydrated phase is not at high enough resolution to give information on the conformation of the polyphenolic head group, the profiles do provide information on the widths of the bilayer and fluid space between bilayers. The approximately 49 Å separation between electron high density peaks in the profile of DPGG (Fig. 3) is similar to that obtained from phospholipid bilayers having the same hydrocarbon chain composition and no hydrocarbon chain tilt. That is, both fully hydrated DPPE (Fig. 3) and DPPC in the presence of tetradecane (which removes chain tilt) have similar headgroup separations in comparable resolution electron density profiles (McIntosh, 1980). The profile for DPGG (Fig. 3) also displays an extremely narrow and relatively high

electron density spacing between head group peaks from apposing bilayers. The electron density of this interbilayer region is greater than that of the methylene chain region of the profile (Fig. 3). Since the electron density of methylene chains is similar to that of water, this implies that part of the DPGG head group extends into the narrow region of the profile between the bilayers. Thus, both the narrowness and relatively high density of the interbilayer space indicate that there is an extremely small fluid spacing between apposing DPPG bilayers, similar to that seen with DPPE bilayers, but smaller than the fluid space between DPPC bilayers (Fig. 3). This narrow fluid spacing implies that the adhesion energy for DPGG must be quite large, as other lipids with small interbilayer separations have large adhesion energies (Evans and Needham, 1987). The interbilayer attractive interactions that could give rise to this small fluid spacing likely arise from a combination of van der Waals interactions, hydrophobic interactions between the aromatic rings on the gallic acids, and interbilayer H-bonding, perhaps via water bridges.

5. Summary

We have synthesized a diacyl lipid (DPGG) with a polyphenolic head group. When hydrated, at room temperature DPGG forms gel ($L\beta$) phase bilayers that strongly adhere to each. Since polyphenolic compounds such as tannic acid bind and precipitate a variety of lipids, proteins, and polymers, DPGG bilayers are likely to be able to bind and tether proteins and membranes. This will be tested in subsequent studies.

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