Effects of unsaturated fatty acids and triacylglycerols on phosphatidylethanolamine membrane structure

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Abstract Lipid intake in diet regulates the membrane lipid composition, which in turn controls activities of membrane proteins. There is evidence that fatty acids (FAs) and triacylglycerols (TGs) can alter the phospholipid (PL) mesomorphism. However, the molecular mechanisms involved are not fully understood. This study focuses on the effect of the unsaturation degree of the C-18 FAs, oleic acid (OA), linoleic acid and linolenic acid, and their TGs, triolein (TO), trilinolein, and trilinolenin, on the structural properties of phosphoethanolamine PLs. By means of X-ray diffraction and ^{31}P-NMR spectroscopy, it is shown that both types of molecules stabilize the H_{II} phase in 1,2-dielaidoyl-*sn*-glycero-3-phosphoethanolamine (DEPE) liposomes. Several structural factors are considered to explain the correlation between the FA unsaturation degree and the onset temperature of the H_{II} phase. It is proposed that TGs could act as lateral spacers between polar DEPE groups, providing an increase in the effective surface area per lipid molecule that would account for the structural parameters of the H_{II} phase. Fluorescence polarization data indicated a fluidification effect of OA on the lamellar phase. TO increased the viscosity of the hydrophobic core with a high effect on the H_{II} phase.—Prades, J., S. S. Funari, P. V. Escribá, and F. Barceló. **Effects of unsaturated fatty acids and triacylglycerols on phosphatidylethanolamine membrane structure. *J. Lipid Res.* 2003.** 44: **1720–1727.**

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Dietary fat intake gives the energy storage to the cell and also participates in membrane composition and cell functions (1–3). The fat composition of the diet can have an effect on the membrane fatty acid (FA) composition. For example, diets rich in oleic acid (OA), such as the Mediterranean diet, are associated with increases in the levels of this FA in various plasma membrane lipid species in rat and human cells (4–6). Also, many of the unsaturated FAs, such as linoleic acid (LL) and α-linolenic acid (LN), are components of the membrane phospholipids (PLs) and are not produced by human cells. Moreover, the FA composition of membranes influences the localization and activity of G proteins and protein kinase C (7), which are pivotal elements in cell signaling and which control (through these proteins) important physiological functions, such as blood pressure (8).

Triacylglycerols (TGs) constitute the largest source of dietary FAs. It is well known that TG cleavage at the brush border membrane is a prerequisite for efficient FA absorption (9). On the other hand, it has been demonstrated that TGs can partition with a preferred orientation in phosphocholine (PC) lipids, although they are neutral lipids (10–15). These data suggest the possibility that TGs could also be present in small proportions inserted in PL bilayers of biological membranes. In fact, it has been suggested that TGs, as interfacial molecules, could be important in the interaction between the membrane and some lipolytic enzymes and carrier proteins (12, 14). In this case, the intracellular pool of TGs could participate in several cellular events, such as the formation of TG-rich lipoproteins and (transient) functional bilayer structures rich in TG and FA storage.

FAs are major components of membranes, mainly bound to PLs and cholesterol esters, although low levels of free fatty acids (FFAs) can also be found in natural membranes (16) and may be important components in certain membranes, such as the small intestine brush border membrane (17). Membrane FA composition has a modulating effect on protein activities such as receptors, ion channels, second messengers, and gene expression (7,

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Abbreviations: DEPE, 1,2-dielaidoyl-*sn*-glycero-3-phosphoethanolamine; DOPE, 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine; EA, elaidic acid; FA, fatty acid; FFA, free fatty acid; HPE, 1-hexadecanoyl-2-(1-pyrenedecanoyl)-*sn*-glycero-3-phosphoethanolamine; LL, linoleic acid; LN, linolenic acid; OA, oleic acid; PC, phosphocholine; PE, phosphoethanolamine; PL, phospholipid; pyS DHPE, *N*-(1-pyrenesulfonyl)-1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine triethylammonium salt; SA, stearic acid; TG, triacylglycerol; TLL, trilinolein; TLN, trilinolenin; TMA-DPH, 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene *p*-toluenesulfonate; TO, triolein.

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18–21). In addition, there is evidence suggesting the existence of a close correlation between some functions of the cells and the various degrees of unsaturation in the *sn*-2-acyl chains of membrane PLs (22, 23). Also, a recent study has shown strong correlations between membrane PL composition and insulin sensitivity in humans (24).

Phosphoethanolamine (PE) PLs are the main group of PLs in the inner leaflet of mammalian plasma membranes (4), where they are mainly organized into lamellar structures. However, PE PLs are also prone to form nonlamellar structures, such as the inverted hexagonal H_{II} phase (25–29). Some special features, such as the control of functions of membrane proteins and the structural organization inside cells, were attributed to H_{II}-prone PLs (30–33). Under a variety of circumstances, cell membrane lipid composition can be modulated to balance the contents of prolamellar and prononlamellar lipids (7, 34–36). Therefore, changes in lipid composition may represent a membrane adjustment in order to regulate structural properties to preserve their functions [e.g., (35, 36)].

There is evidence that FFAs and TGs can modify the polymorphic properties and fluidity of PLs in model membranes (10–12, 15, 37–40). However, the molecular mechanisms involved in the modulation of the membrane structure and function are not fully understood. Therefore, the study of structural properties of PL membranes in which FFAs or TGs are present is important for a better understanding of their crucial biological function. The present work was planned to analyze the effect of the unsaturation in the C-18 acyl chain of the FAs, (OA, LL, and LN) and their sterified derivatives, such as TGs [triolein (TO), trilinolein (TLL), and trilinolenin TLN)], on the structural properties of lamellar and nonlamellar PE PLs, as model membranes.

MATERIALS AND METHODS

Materials

1,2-Dielaidoyl-*sn*-glycero-3-phosphoethanolamine (DEPE) and 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE) were purchased from Avanti Polar Lipids, Inc. (Alabaster, U.S.A.). 1-Hexadecanoyl-2-(1-pyrenedecanoyl)-*sn*-glycero-3-phosphoethanolamine (HPE), *N*-(1-pyrenesulfonyl)-1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine triethylammonium salt (pyS DHPE), and 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene *p*-toluenesulfonate (TMA-DPH) were from Molecular Probes (Leiden, The Netherlands). OA (*18:1c*Δ*9*), LL (*18:2c,c*Δ*9,12*), α-LN (*18:3c,c,c*Δ*9,12,15*), TO, TLL, TLN, and *N*-(2-hydroxy ethyl) piperazine-*N*′-(2-ethanesulfonic acid) sodium salt (HEPES) were obtained from Sigma Chemical Co. (Madrid, Spain). Lipids, FAs, and TGs were stored under argon at −80°C until use. Differential scanning calorimetry (DSC) of multilamellar liposomes from these phosphatidylethanolamine derivatives was used to evaluate the PL purity. DSC calorimetric scans showed highly cooperative phase transitions.

Sample preparation

Multilamellar lipid vesicles 15% (w/w) were prepared in 10 mM HEPES, 100 mM NaCl, 1 mM EDTA, pH 7.4 (HEPES buffer) for X-ray studies, or in D_2O for NMR experiments. PLs with FAs or TGs were prepared at a molar ratio of 20:1 (PL-fat). Lipid mixtures were thoroughly homogenized with a pestle-type minihomogenizer (Sigma) and vortexed until a homogeneous mixture was obtained. Then the suspensions were submitted to five temperature cycles (heated up to 70°C and cooled down to 4°C). Samples for X-ray scattering experiments were stored at −80°C under argon and allowed to equilibrate at 4°C for 48 h before measurements. Samples for NMR experiments were equilibrated at 4°C for at least 24 h prior to data acquisition. For fluorescence spectroscopy experiments, PL and fluorophore (PL-probe; 1,000:1; mol/mol), in the presence or absence of OA or TO (PL-fat; 20:1; mol/mol), were dissolved in chloroform-methanol (2:1; v/v), evaporated under argon, and vacuum-dried for at least 3 h. The lipid film was resuspended in HEPES buffer by vortex shaking at ~45°C. The lipid suspension (120 μM) was subjected to five freeze/thaw cycles to ensure the complete hydration of the lipid vesicles. To obtain large unilamellar vesicles (LUVs), the resulting multilamellar suspension was passed 11 times through polycarbonate membranes (0.1 μm) in an extruder (Avanti Polar Lipids, Inc.). LUVs were used immediately.

X-ray diffraction

Small- and wide-angle (SAXS and WAXS) synchrotron radiation X-ray scattering data were collected simultaneously, using standard procedures on the Soft Condensed Matter beamline A2 (41, 42) of Hasylab at the storage ring DORIS III of the Deutsches Elektronen Synchrotron. Data were acquired continuously for 15 s at each temperature, followed by a waiting time of 45 s with a local shutter closed. During data collection, samples were heated from 27°C to 75°C at a scan rate of 1°C/min. Then they were kept at the highest temperature for 5 min and finally cooled down to the lowest temperature at the same scan rate. The experimental conditions did not affect the phase sequence structures or their parameters. Positions of the observed peaks were converted into distances, *d*, after calibration with the standards rat tendon tail and poly-(ethylene therephtalate) for the SAXS and WAXS regions, respectively. Interplanar distances, d_{hkl}, were calculated according to equation 1:

$$s = 1/d_{hkl} = (2\sin\theta)/\lambda \qquad \textit{(Eq. 1)}$$

where *s* is the scattering vector, 2θ is the scattering angle, λ (0.154 nm) is the X-ray wavelength, and *hkl*s are the Miller indexes of the scattering planes.

^{31}P-NMR

Measurements were conducted on a model AMX-300 multinuclear NMR spectrometer (Bruker Instruments) in 4 mm tubes. Samples were equilibrated at the working temperatures for 15 min before data acquisition. ^{31}P-NMR free induction decays were accumulated for up to 160 transients by employing a 6.75 μs 90° radio frequency pulse, 12.2 kHz sweep width, and 32 K data points. The delay between transients was 2 s. The spectra were obtained by scanning from lower to higher temperatures.

Fluorescence polarization

Experiments were done with a MPF-66 fluorescence spectrophotometer (Perkin-Elmer). The cuvette was thermostated during the experiment. LUV suspensions, containing the fluorophores HPE or pyS DHPE, were excited at 340 or 350 nm, and fluorescence polarization was registered at 378 or 379 nm, respectively. TMA-DPH was excited at 360 nm, and the fluorescence emission was recorded at 427 nm. The bandpass was 4 nm for excitation and for emission. Samples were equilibrated for 5 min at each temperature, prior to measurement. In our experimental conditions, the inner filter effect became critical when sample

absorption was ~0.1. Thus, lipid samples were diluted until the fluorescence samples had an absorption at the excitation wavelength of ~0.04. Light scatter was checked by a control experiment with unlabeled liposomes. A residual fluorescence (~5% for DOPE and 4% for DEPE) was observed in the control experiments with unlabeled liposomes, and its contribution to the fluorescence polarization was insignificant. Fluorescence polarization was calculated according to equation 2:

$$P = (I_{VV} - GI_{VH}) / (I_{VV} + GI_{VH}) \quad \textit{(Eq. 2)}$$

where I_{VV} and I_{VH} are fluorescence intensity values measured with the excitation and emission polarizers in parallel and perpendicular, respectively. G is an instrumental factor.

RESULTS

X-ray diffraction

Figure 1 illustrates the SAXS and WAXS X-ray scattering patterns of DEPE alone or in the presence of the FA, LN, or the TG, TLN, both at a molar ratio of 20:1 (DEPE-fat). The sequence of diffraction patterns collected showed defined phase transitions that allowed unequivocal characterization of the structures and their respective lattice parameters. The L_β phase was identified by a sharp and intense SAXS reflection ($s \approx 0.15$ nm^{-1}), accompanied by a reflection in the WAXS region. The L_α phase was identified by a single reflection peak at $s \approx 0.19$ nm^{-1}, with a very good signal-to-noise ratio. The appearance of three diffraction orders in the SAXS region with a *d* spacing ratio of $1:1/\sqrt{3}:1/\sqrt{4}$ indicated the formation of the H_{II} phase. All the samples showed similar X-ray scattering patterns.

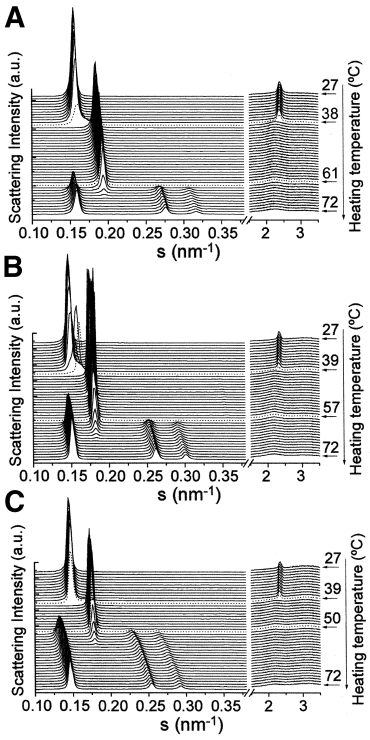

Fig. 1. Sequence of SAXS (left) and WAXS (right) scattering patterns of (A) 1,2-dielaidoyl-*sn*-glycero-3-phosphoethanolamine (DEPE), (B) DEPE with α-linolenic acid (LN), and (C) DEPE with trilinolenin (TLN) at a molar ratio of 20:1. The heating sequence is shown from 27°C to 72°C. The scan rate was 1°C/min. Successive diffraction patterns were collected for 15 s every min. The thermal sequence of the phases L_β (up to 39°C), L_α (from 39°C to 57°C for DEPE-LN and from 39°C to 50°C for DEPE-TLN), and H_{II} (up to 72°C) is clearly shown. The phase transition from L_β to L_α can be identified by the vanishing peak on the WAXS region of the pattern.

Effects of the FA unsaturation on DEPE membrane structures

DEPE alone and in the presence of the unsaturated FAs OA, LL, and LN showed a phase sequence from gel L_β to liquid crystalline L_α and to hexagonal H_{II} phases with increasing temperature (**Fig. 2A–C**).

Lamellar phases

DEPE-FA (20:1; mol/mol) samples showed an L_β phase up to 39°C with a constant repeat distance of ~5.4 nm (**Table 1**), similar to the value shown for DEPE alone and previously reported (37). Nevertheless, the presence of the FAs OA, LL, and LN strongly destabilized the DEPE L_α phase (Fig. 2A–C). Although the temperature range of the L_α phase presence was dependent upon the number of unsaturations of the acyl chain (Table 1), the repeat distance decreased linearly with temperature and showed a constant value (~−0.013 nm/°C) for the compression coefficient (Table 1).

DEPE-OA mixtures were characterized by X-ray diffraction measurements (37). It was observed that the temperature range in which the lamellar and nonlamellar phases coexisted depended on the DEPE-OA molar ratio. For ex-

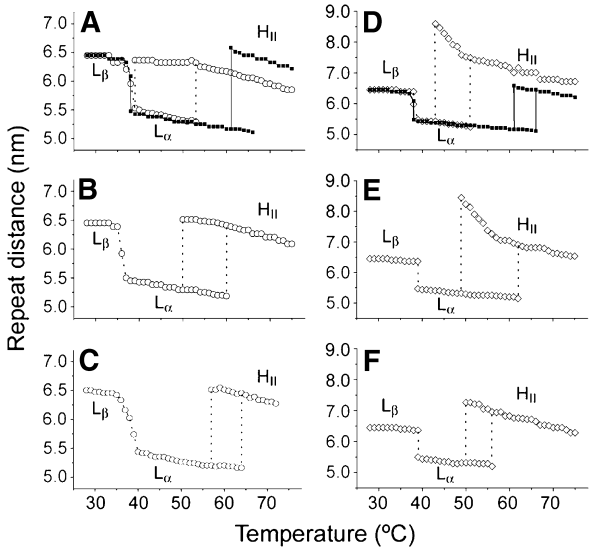

Fig. 2. Dependence of the interplanar repeat distance *d* on temperature for DEPE alone (solid squares in A and D) and in the presence of the free fatty acids (A) oleic acid (OA), (B) linoleic acid, and (C) LN (open circles), or the triacylglycerols (D) triolein (TO), (E) trilinolein, and (F) trilinolenin (open diamond). Sample concentration was phospholipid (PL)-fat, 20:1 (mol/mol). Phases represented are: L_β, L_α, and H_{II}. The coexistence of the $L_\alpha + H_{II}$ two-phase region is indicated by the interval temperature range shown by the vertical lines.

TABLE 1. Summary of the physical (structural) properties

Composition	$\delta d/\delta T\ (L_\alpha)^a$	$\delta d/\delta T\ (H_{II})^a$	$\Delta T_{L\alpha}{}^b$	$d_{L\alpha}{}^d$	$d_{HII}{}^d$
	nm/°C	*nm/°C*	*°C*	*nm*	*nm*
DEPE	−0.012	−0.024	38–61(66)	5.43	6.27
DEPE-OA[c]	−0.014	−0.020	(38) 39 (53)	5.44	5.90
DEPE-LL	−0.012	−0.027	(33)37–50(60)	5.40	6.15
DEPE-LN	−0.017	−0.021	(36)39–57(64)	5.40	6.20
DEPE-TO	−0.017	−0.036	38–43(51)	5.43	6.72
DEPE-TLL	−0.012	−0.039	(39)40–49(62)	5.43	6.58
DEPE-TLN	−0.013	−0.037	39–50(56)	5.44	6.45

DEPE, 1,2-dielaidoyl-*sn*-glycero-3-phosphoethanolamine; OA, oleic acid; LL, linoleic acid; LN, linolenic acid; TO, triolein; TLL, trilinolein; TLN, trilinolenin. Sample DEPE-FFA and DEPE-TG composition was 20:1 (molar ratio). The angular coefficient of the dependence of the interplanar distance d_{10} on the temperature $\partial d/\partial T < 0$ indicates a compression process.

[a] The compressibility of L_α is linear in the single or two-phase regions. Single H_{II} phases have linear compressibility factor. The temperature range in which the L_α phase is observed is shown in $\Delta T_{L\alpha}$.

[b] The parentheses indicate the temperature limit of the L_α phase in a two-phase region. Values on the left correspond to $L_\beta + L_\alpha$ and on the right to $L_\alpha + H_{II}$ temperature range coexistence.

[c] No single L_α phase was observed.

[d] $d_{L\alpha}$ at 40°C and d_{HII} at 72°C.

ample, in the mixture DEPE-OA (20:1; mol/mol), the L_α phase was observed from 38°C to 53°C and in coexistence with either L_β or H_{II} phases (Table 1). DEPE in the presence of LL or LN (20:1; mol/mol) showed a single L_α phase in the range of 37–50°C or 39–57°C, respectively, with a repeat distance value of 5.4 nm at 40°C. Above these temperatures, lamellar (L_α) and hexagonal (H_{II}) phases coexisted in a range that depended also on the FA (Fig. 2B, C and Table 1).

Hexagonal H_{II} phases

The threshold temperature for DEPE-based H_{II} phases formed by the binary system (DEPE-FFA; 20:1; mol/mol) depended on the unsaturation degree of the FA (Fig. 2A–C). The temperature at which a single inverted hexagonal phase appeared was directly related to the degree of unsaturation of the FA. It is important to note that the repeat distance of the DEPE H_{II} phase (6.27 nm at 72°C) was reduced by the FA OA (5.90 nm at 72°C), while LL and LN produced a smaller effect (6.15–6.20 nm at 72°C). The compressibility factor (∼−0.024 nm/°C) did not change in a significant mode in the presence of an unsaturated FFA at a molar ratio of 20:1, suggesting a similar packing of the PL molecules in the DEPE and DEPE-FFA systems.

Effects of TGs on DEPE membrane structures

DEPE in the presence of the TGs TO, TLN, and TLL had the same phase sequence pattern, L_β to L_α to H_{II}, as DEPE in presence of FFAs (Fig. 1). The diffraction patterns of the DEPE-TG samples (Figs. 2D–F) showed several important general trends: *i*) TGs, like FFAs, did not alter the interplanar distance of the L_α phase (5.4 nm at 40°C), or the compressibility factor ($\partial d/\partial T \approx -0.012$) (Table 1). *ii*) TO, TLL, and TLN facilitated formation of the H_{II} phase, and there was a long range of temperatures where L_α and H_{II} phases coexisted (∼6°C to 13°C) (Figs. 2D–F). The spacing of the hexagonal phase started at a high value (∼9 nm), showed a strong decay during the coexistence of the L_α and H_{II} phases, and then followed the pattern of the DEPE-FFA systems. *iii*) TGs increased the intercylinder distance of the DEPE H_{II} phases. The lattice spacing was in the range of 6.7–6.4 nm for DEPE-TG (20:1, molar ratio) and 6.27 nm for DEPE liposomes (data taken at 72°C; Table 1). In addition, the negative value of the compressibility factor decreased ($\partial d/\partial T \approx -0.037$ nm/°C) compared with DEPE alone ($\partial d/\partial T \approx -0.024$ nm/°C) (Table 1). Also, notice that for DEPE-TG, the hexagonal (H_{II}) phase contraction factor values are about three times greater than that of the lamellar L_α phase ($\partial d/\partial T \approx -0.013$ nm/°C) (Table 1).

^{31}P-NMR data

The phase behavior of DEPE in the presence of OA or TO was also analyzed by ^{31}P-NMR, working under quasiequilibrium conditions, with a 15 min rest at each temperature. The spectra showed line shapes characteristic of L_α and H_{II} phases (**Fig. 3**). OA and TO lowered the onset transition temperature of the H_{II} phase with different degrees of cooperation, in agreement with the X-ray diffraction data. DEPE underwent a lamellar-to-inverted hexagonal phase transition between 45°C and 50°C, and above 52°C, a single H_{II} phase was observed. The systems DEPE-OA and DEPE-TO (molar ratio 20:1) had no pure L_α phase. OA showed the highest degree of cooperativity and the H_{II} structure appeared at 42°C. TO presented a lamellar-hexagonal transition in the range of 37–42°C, and from 47°C upward, a single H_{II} phase was observed.

Steady-state fluorescence polarization measurements

DEPE and DOPE were used to assess the effect of the presence of OA and TO on the properties of the lamellar and hexagonal (H_{II}) phases. The fluorescent probe HPE is localized in the hydrophobic core of the membrane and provides structural information on this region. The probes pyS DHPE and TMA-DPH allow monitoring of the lipid environments close to the bilayer surface; pyS DHPE is anchored in close proximity to the bilayer surface, and

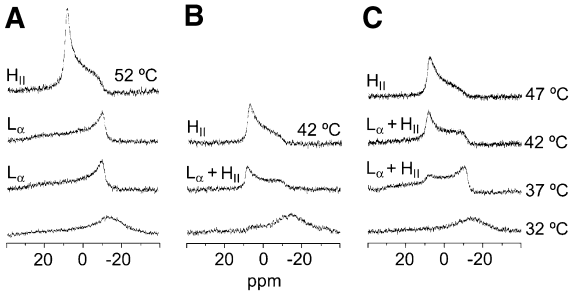

Fig. 3. ^{31}P-NMR spectra of DEPE aqueous dispersions (15%, w/w) in D_2O, as a function of temperature. A: DEPE alone and in the presence of (B) OA or (C) TO PL-fat, 20:1; mol/mol). Samples were equilibrated for 15 min at each temperature before data acquisition. Scans were done from lower to upper temperature. The temperatures at which the systems had L_α, $L_\alpha + H_{II}$, and pure H_{II} phases are indicated. Note that DEPE showed the H_{II} phase at 52°C, DEPE-OA at 42°C, and DEPE-TO at 47°C.

TMA-DPH anchors its nonfluorescent polar moiety in the lipid/water interface and interacts with both the PL polar headgroups and the first part (C8–C10) of the fatty acyl chain region (43, 44). The fluorescence polarization of HPE-labeled DEPE liposomes in the presence and absence of OA and TO showed the same trend (**Fig. 4A**), with an apparent gel-to-liquid crystalline transition temperature of ~30–35°C, which was not affected significantly by the presence of fats. OA induced a decrease and TO an increase in the HPE-probe polarization in the lamellar phase, L_β and L_α. However, both types of molecules produced an increase of the fluorescence polarization of HPE-labeled DOPE liposomes, although TO had a significantly higher effect (Fig. 4C). DOPE has an H_{II} structure under the experimental conditions; thus, one expects that increasing the temperature will increase the molecular Brownian movement and therefore will decrease the polarization of the probe. Nevertheless, fluorescence polarization data showed an opposite trend, more acute in the DOPE-OA sample. This peculiar property could be explained considering the X-ray diffraction data of DOPE-OA (20:1; mol/mol) reported previously (37), which showed a value for the thermal compression factor (−0.014 nm/°C) significantly lower than for DOPE alone (−0.021 nm/°C), indicating a tighter packing of the lipid molecules with the temperature, which would hinder the rotational movement of the probe. In the other region of the membrane, OA and TO showed a similar effect. The fluorescence polarization of TMA-DPH-labeled DEPE (Fig. 4B) and pyS DHPE-labeled DOPE (Fig. 4D) membranes increased with a higher value for the probe pyS DHPE.

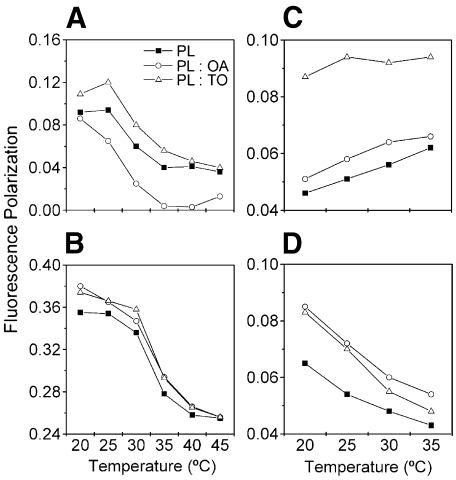

Fig. 4. Fluorescence polarization versus temperature of 1-hexadecanoyl-2-(1-pyrenedecanoyl)-*sn*-glycero-3-phosphoethanolamine (HPE)-labeled DEPE (A), 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene *p*-toluenesulfonate-labeled DEPE (B), HPE-labeled 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE) (C), and *N*-(1-pyrenesulfonyl)-1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine triethylammonium salt (pyS DHPE)-labeled DOPE (D) liposomes, in the absence or presence of OA or TO. Sample composition was: probe-phospholipid (PL), 1:1000 (mol/mol); PL-OA and PL-TO, 20:1 (mol/mol).

DISCUSSION

It has been demonstrated that the composition of some biological membranes is influenced by dietary fats (4–6, 22). Among the biologically unsaturated FAs, the C-18s are important in the fat composition of the Mediterranean diets. Thus, considering the importance and influence of diet on membrane properties, we focused our interest on the effect of unsaturated fats, which are an essential component of biological membranes, on the structural properties of PE membranes.

From the experimental data reported, it seems quite plausible that membranes can incorporate small amounts of FFA and TG into the bilayer structure, in agreement with previous reports (10–15, 37–40). But, to our knowledge, this is the first detailed study of the thermotropic phase behavior of PE PLs and TGs. Interestingly, the unsaturated FAs (OA, LL, and LN) and their respective TGs (TO, TLL, and TLN) induced a marked decrease of the L_α-to-H_{II} phase transition temperature in DEPE lipids (Figs. 1, 2). In addition, ^{31}P-NMR measurements done under quasiequilibrium conditions showed that TO, like OA, favored the formation of the H_{II} nonlamellar phase (Fig. 3). Thus, the main effect of the unsaturated FFAs and their TGs would be to stabilize the H_{II} phase, although they showed a differential relative efficiency in the perturbation of the lipid phase properties in the lamellar and inverted hexagonal structures.

FAs

The X-ray diffraction data of the FFAs in DEPE liposomes showed that the presence of one, two, or three *cis* double bonds in the acyl chain, such as in OA, LL, and LN, drastically affected the temperature range of the L_α phase presence, as a unique phase or in coexistence with the nonlamellar H_{II} phase (Table 1). One interesting observation is that the increase in the degree of unsaturation of the FA contributes to increases in the temperature range of existence of the lamellar L_α phase. Also, the onset temperature of a single hexagonal phase was directly related to the degree of unsaturation of the FA. Thus, the interval of temperatures where lamellar and nonlamellar phases coexisted depended on the FA. In addition, the C-18 unsaturated FAs did not alter the lamellar bilayer thickness and had a relatively minor effect on the H_{II} lattice spacing (d = 6.27 nm for DEPE alone, 5.90 nm in the presence of OA, and ≅ 6.2 nm in presence of LL and LN).

From the structural point of view, several factors could influence the ability of these molecules to determine the lipid phase propensity. A DSC study of FAs with varying degrees of unsaturation demonstrated the importance of the pH in controlling the degree of carboxyl ionization of the FA and the electrostatic repulsions among the bilayers (38). It is reported that the pK of FAs is quite insensitive to the unsaturation degree [LL had a pK value 0.1 units higher than stearic acid (SA)]; thus, the ionization state of the C-18 FAs would be about the same, independent of the FA structure. However, the DSC data obtained for the series C-18 and C-22 at pH 7.4 point to the fact that struc-

tural factors such us unsaturation and length affect the hexagonal phase-forming ability of the FFAs. Our X-ray diffraction data are in agreement with these previous DSC results with the series C-18; the increase in the unsaturation degree of FAs or their TGs correlates with an increase in the L_α-H_{II} transition temperature (Table 1 and Fig. 2).

A comparative X-ray diffraction analysis at pH 7.4 of the effect of the C-18 FAs, OA, elaidic acid (EA), and SA showed the importance of the steric disturbance induced by the FA molecular shape on the acyl-chain moiety of the PE membrane (37). From these results, a *cis* double bond (such as OA) would bring the greatest disruption of the lamellar phase, while the presence of a trans double bond (EA) or the absence (SA) affected slightly the H_{II} propensity of PE membranes. These data could be explained by the lipid-packing theory (45). Another parameter could be the molecular location of FAs within the membrane, which could be influenced by structural properties. Some works have provided evidence;. e.g., spin label electron paramagnetic resonance experiments on 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine/myristic acid (1:2 molar ratio) mixtures showed that the FA acyl chain was located approximately one methylene group deeper than the *sn*-2 chain of the PC in the H_{II} phase (46). FAs labeled with spectroscopic probes showed differences between the depth of FAs and the PC fatty acyl chains (44). In addition, the carboxyl ionization decreased FA depth in membranes (47). In our system, the degree of unsaturation could affect the depth of the C-18 FAs in the membrane. Differences in depth could modify the hydrophobic/hydrophilic balance in DEPE-FA membranes, reducing the DEPE headgroup area relative to its volume and increasing the intrinsic monolayer curvature. In this case, the H_{II} phase would be induced at lower temperatures.

TGs

The X-ray diffraction data showed that TG, TO, TLL, and TLN were incorporated into DEPE liposomes. Their gel and liquid-crystalline lamellar phases had similar properties, and the structural parameters, such as the lattice spacing and the compressibility factor, were not affected by the nature of the TG molecule (Figs. 2D–F and Table 1). Therefore, there should be little discrimination between the different TG species incorporated into a lamellar phase of a biological membrane. It was proposed that TO incorporated into PC bilayers had the carbonyl groups oriented toward the aqueous interface, with the 1- and 3-carbonyls slightly closer to the aqueous interface than the 2-carbonyl (11, 14). Extrapolating this possible model to our PE systems, TO would be integrated in the membrane with the polar headgroup and the acyl chains oriented parallel to the PE molecules. We propose that the presence of the bulky TG molecules in the lamellar DEPE liposomes increases the monolayer bending to force the kinetic formation of an H_{II} structure with a high intercylinder distance (Figs. 2D–F). As the temperature increases, the interface bending provides for an approach of lipid headgroups and forces the slightly polar TG molecule depth into the hydrocarbon matrix. TG homogenization in the membrane will reduce the acyl chain stress up to the final H_{II} structure. TGs could be considered to act as lateral spacers between polar DEPE groups, providing an increase in the effective surface area per lipid molecule and in the diameters of the water cylinders. Also, differences in the decrease in the cross-sectional area of DEPE and TG molecules upon dehydration, as the temperature increases, could be responsible for the high negative value of the compressibility factor (Table 1). The structural differences among the different molecules, TO, TLL, and TLN, mainly due to the number of double bonds per acyl chain, would result in differences in the intercylinder distances (Table 1).

Fluorescence polarization data

The effect of the FAs or the TGs on the properties of the lamellar and H_{II} phases was analyzed using OA or TO as references. Both types of molecules had a reduced effect on the fluorescence polarization of the probes located in the upper region of the membrane. Their differences came from the hydrophobic acyl region. OA notably increased the fluidity of the acyl chain region in the gel and in the liquid-crystalline lamellar phases (Fig. 4A). An increase in the fluidity would favor a diffusion process across the membrane. Consequently, an important role for the unsaturated FFAs in the biological membranes could be to create a dynamic lipid environment to allow a rapid lateral diffusion of transient molecules within the hydrophobic region of the lipid bilayer. However, OA had a slight effect on the fluorescence polarization of the HPE probe in the DOPE H_{II} structure ($\sim$10%) (Fig. 4C). Because the polarization of the probe depends on the rotational mobility and the degree of restriction of its motion, these results suggest that the increase in fluidity induced by OA is probably counteracted by an increase in the acyl chain packing. Note that DOPE samples showed an increase in the polarization value with the temperature, probably due to a higher packing of the lipid molecules, as was reported in X-ray diffraction studies with DOPE (48) and DOPE-OA (37). Therefore, the rotational movement of the HPE probe would be hindered as the temperature increased. On the other hand, TO slightly increased the HPE fluorescent polarization in the lamellar phase ($\sim$20% at 37°C) and had a main effect in DOPE H_{II} structures ($\sim$85% at 20°C), probably due to an increase in the microviscosity of the acyl surface. Considering the model proposed from the X-ray diffraction data, if the H_{II} phase stabilization by TG molecule is accompanied by an increase in the depth position of the TG molecule in the lipid matrix, one would expect to get an increase in the HPE polarization, as we observed.

In summary, the incorporation of an unsaturated FA or a TG into a PE membrane would facilitate the L_α-H_{II} phase transition. However, the temperature range of the presence of the L_α phase was related to the degree of unsaturation of the FA. Although it is rather improbable that TGs are incorporated into cell membranes, it is important to note that their presence in PE liposomes would favor the kinetic process of the transition L_α to H_{II}. The reported data indicate the possibility that the membrane

lipid composition could be modulated, e.g., by the FFA composition and/or appropriate metabolic adjustments, to counteract a necessity, such as to preserve the lamellar liquid crystalline phase essential to membrane stability and functions, or facilitate the formation of a transient microdomain prone to an H_{II} structure.

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