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Nature of the Charged Headgroup Determines the Fusogenic Potential and Membrane Properties of Lithocholic Acid Phospholipids

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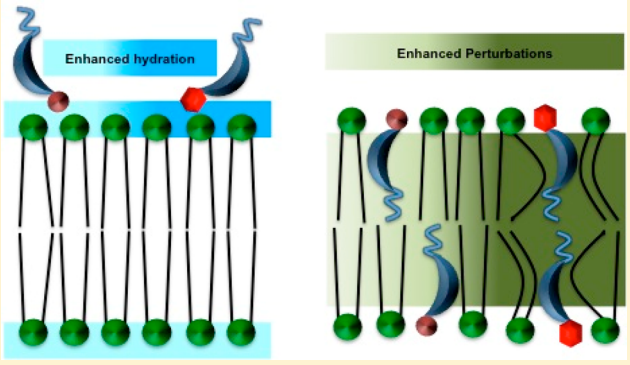
ABSTRACT: Phospholipids play a crucial role in many cellular processes ranging from selective membrane permeability, to membrane fission and fusion, to cellular signaling. Headgroups of phospholipids determine the membrane properties and fusogenicity of these lipids with target cell membranes. We studied the fusogenic and membrane properties of phospholipids possessing unnatural charged headgroups with model membranes using laurdan based membrane hydration studies, DPH based membrane fluidity, and differential scanning calorimetry. We unravel that fusogenicity, membrane hydration, and fluidity of membranes are strongly contingent on the nature of the phospholipid charged headgroup. Our studies unraveled that introduction of bulky headgroups like dimethylamino pyridine induces maximum membrane hydration and perturbations with high fusogenicity as compared to small headgroup based phospholipids. These phospholipids also have the capability of high retention in DPPC membranes. Hydration and fluidity of these phospholipid-doped DPPC membranes are contingent on the nature of the charged headgroup. This study would help in future design of phospholipid based nanomaterials for effective drug delivery.



INTRODUCTION

Mammalian cell membranes are composed of a range of phospholipids like phophatidylinositol, phosphatidic acid, sphingomyelin, phosphatidylserine, and phosphatidyl ethanolamine with varying headgroups.[1] Phospholipids depending on their molecular architecture play an important role in membrane organization, selective permeability, membrane fusion, and cellular signal transduction.[2] Therefore, local lipid composition and nature of the charged headgroup on these phospholipids play a critical role in formation of endocytic/exocytic vesicles and membrane fusion processes.[3] Fusogenic properties of phospholipids are strongly contingent on acyl chain length, degree of unsaturation, sterol content in membranes, and dynamics of polar headgroup.[4] Physicochemical events involved in fusion of these lipid molecules are poorly understood due to complex nature of lipid compositions in cellular membranes.

A large number of functional materials have been engineered for drug delivery, tissue engineering, gene therapy, sensing, and implant applications.[5−9] These engineered materials with insufficient biocompatibility can be recognized from their surface as foreign material by immune system. Therefore, engineering of biocompatible materials is essential to prevent these catastrophic results.[10] Naturally occurring and synthetic phospholipids provide attractive materials for biomedical applications as they can circumvent the activation of immune system.[11]

Intracellular delivery of nanomaterials/drugs encounters electrostatic binding with the cellular surface, followed by membrane fusion involving hydrophobic interactions with lipid acyl chains.[12] Nunes et al. unraveled the dependency of drug−membrane interaction on degree of drug ionization and lipid phase state.[13] As organization and function of membranes is contingent on dynamics of the confined water,[14] Daniele et al. showed distribution of pH sensitive lysine based cationic surfactant in biomembranes.[15] Interaction of 5-fluorouracil as free drug and its nanoparticles with model membranes suggested that free drug follows concentration-dependent reversible diffusion whereas nanoparticles absorbed on the membrane of DMPC result in continuous delivery of the drug.[16] Brian et al. showed the effect of mismatch of acyl chains on membrane organization.[17]

Bile acids present interesting materials for biomedical applications due to (a) their facial amphiphilic,[18] (b) high biocompatibility, (c) existence of diverse chemical scaffolds, and (d) presence of reactive functional groups.[19,20] Bile salts at submiceller concentrations are known to induce hydration of

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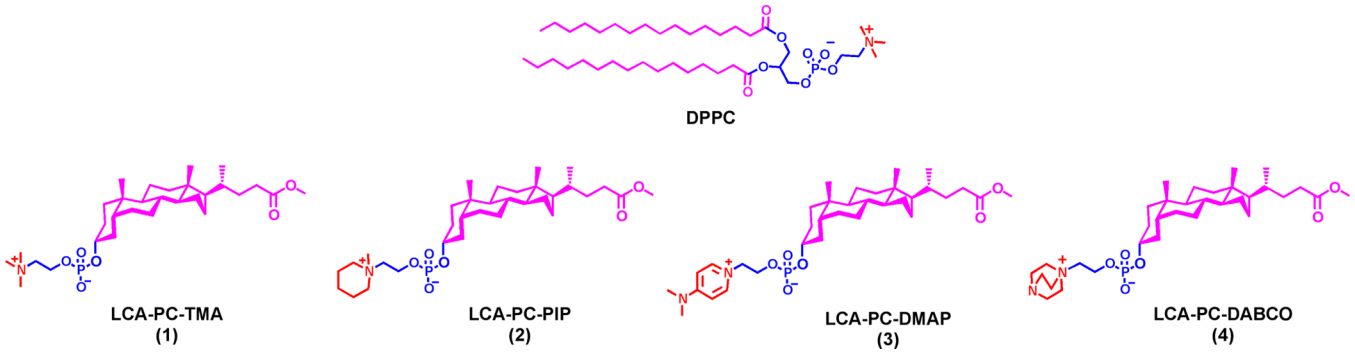

Figure 1. Chemical structures of DPPC and lithocholic acid based phospholipids with different charged headgroups.

model membranes.[21] Recently, we have shown that the presence of hydroxyl groups at C_7 and C_{12} positions on bile acid phospholipids essentially drives their self-assemblies in membranes.[22] Therefore, engineering and interactions of bile acid based materials with biomembranes would be critical for design of new nanomaterials for biomedical applications.[23]

The nature of the charged headgroups on lipids plays a critical role in antibacterial,[24] anticancer,[25] sensing,[26] drug delivery,[27] and gene transfection[28] applications of nanomaterials. Antibacterial activities of charged amphiphiles have shown that multiheaded pyridinium amphiphiles were more active compared to their trimethylammonium counterparts.[29] Gold nanoparticles tuned with different charged headgroups were shown to detect and differentiate between different cancer cell types due to preferential binding of these particles for a specific cell type.[30] Bhattacharya and co-workers have demonstrated that headgroup chemistry of cationic lipids dictates serum compatibility and transfection efficiencies of cholesterol based cationic lipids.[31]

In this article, we hypothesize that fusogenic properties of phospholipids with model membranes, and their interactions with natural phospholipids (DPPC) in vesicular membranes, are contingent on the nature of the charged headgroup. Therefore, we used lithocholic acid (LCA) based phospholipids possessing unnatural charged headgroups like small trimethyl amine (LCA-PC-TMA), aliphatic hydrophobic *N*-methylpiperidine (LCA-PC-PIP), aromatic *N*,*N*-dimethylamino pyridine (LCA-PC-DMAP), and 1,4-diazabicyclo[2.2.2]octane (LCA-PC-DABCO) (Figure 1). We studied fusogenic properties of these lipids and their membrane properties using laurdan based hydration, diphenylhexatriene (DPH) based fluorescence anisotropy, and differential scanning calorimetry (DSC) studies.

EXPERIMENTAL METHODS

Materials. 1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) was obtained from Avanti Polar Lipids, and DPH and laurdan were obtained from Sigma-Aldrich.

Preparation of Vesicles. Neat DPPC membranes or coliposomes of DPPC membranes with LCA phospholipids were prepared according to published protocols.[23]

Laurdan Based Hydration Studies.[32] Laurdan doped DPPC membranes (DPPC:laurdan 100:1) were prepared with and without doping of LCA phospholipids at different concentrations (10, 30, 50, 70 mol %). For incubation studies, DPPC vesicles containing laurdan were incubated with 10, 20, 30 mol % of LCA phospholipids. GP (generalized polarization) was measured at every 6 h interval in a 96-well plate using a Molecular Device M5 instrument using λ_{ex} of 350 nm and λ_{em} of 440 and 490 nm.[32] General polarization (GP) was calculated using eq 1 as described in earlier studies.[32]

$$GP = (I_{440} - I_{490})/(I_{440} + I_{490}) \quad (1)$$

Temperature dependent generalized polarization studies were performed on LCA phospholipid doped membranes. All experiments were performed at least two times in duplicates, and results are expressed in form of mean ± SD.

Fluorescence Anisotropy Studies.[33] DPH doped DPPC membranes were prepared in a similar way as mentioned above with incorporation of DPH (1/100 of DPPC). The anisotropy experiment was done in a 96-well plate with λ_{ex} at 350 nm and λ_{em} of 452 nm. Steady state fluorescence anisotropy (r_{ss}) was then calculated using eq 2 as described in earlier studies.[33]

$$r_{ss} = (I_{\parallel} - GI_{\perp})/(I_{\parallel} + 2GI_{\perp}) \quad (2)$$

$I_{\parallel}$ and $I_{\perp}$ are emission intensities in parallel and perpendicular directions to plane of excitation. G is an instrument-specific factor measured to correct instrument polarization, which is equal to $I_{\perp\parallel}/I_{\perp\perp}$.

For incubation studies, DPH doped DPPC membranes were incubated with 10, 20, 30 mol % of LCA phospholipids, and anisotropy was measured at every 6 h interval. For studying membrane properties of LCA phospholipid doped membranes, we performed temperature dependent anisotropy studies. All experiments were performed at least two times in duplicate and results expressed in the form mean ± SD.

Differential Scanning Calorimetry. DSC studies have been performed on Nano DSC, TA Instruments, using LCA phospholipid doped DPPC membranes as described in earlier studies.[34] All thermodynamic parameters were computed using Nanoanalyze and OriginPro 8.5.1 software.[34] Size of cooperativity unit (CU) was measured for phase transition using formula 3

$$CU = \Delta H_{vH}/\Delta H_c \quad (3)$$

where ΔH_{vH} is van't Hoff enthalpy and ΔH_c is calorimetric enthalpy.

Van't Hoff enthalpy is expressed in terms by formula[35] 4

$$\Delta H_{vH} = (X)(4RT_m{}^2C_p{}^{max})/(\Delta H_c) \approx (X)(6.9T_m{}^2/\Delta T_{1/2}) \quad (4)$$

where ΔH_{vH} depends on mole fraction of doped or incubated drug, (X) is mole fraction component, ΔH_c is calorimetric enthalpy, T_m is transition temperature, and $C_p{}^{max}$ is specific heat capacity.

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RESULTS AND DISCUSSION

Fusogenic Properties of Phospholipids. A series of physicochemical events involved during lipid fusion to cell membranes comprises electrostatic interactions of donor with acceptor membranes followed by interaction with lipophilic acyl chains of lipid membranes. Balance of these electrostatic and hydrophobic interactions is critical, as it dictates fusogenic potential of lipids. These interactions induce initial change in surface hydration followed by change in rigidity of membranes. Therefore, we studied change in hydration and rigidity of model membranes on incubation with different (10, 20, 30) mol % of LCA phospholipids.

For laurdan based hydration studies, we initially investigated the change in surface hydration of DPPC membranes on incubation with LCA phospholipids using laurdan as a fluorescence probe. Laurdan emission is sensitive to interfacial hydration of membranes and therefore would reflect on interfacial fusion on incubation of LCA phospholipids with membranes. Laurdan emission is blue-shifted in gel-like state and red-shifted in liquid-crystalline state, which is quantified by generalized polarization (GP). Increase in hydration of membrane surface would induce red shift in the laurdan emission, thereby causing a decrease in GP, whereas membrane dehydration would enhance GP by inducing blue shift in the laurdan emission.[36]

As shown in Figure 2, incubation of LCA-PC-TMA does not induce any appreciable change in GP of DPPC membranes

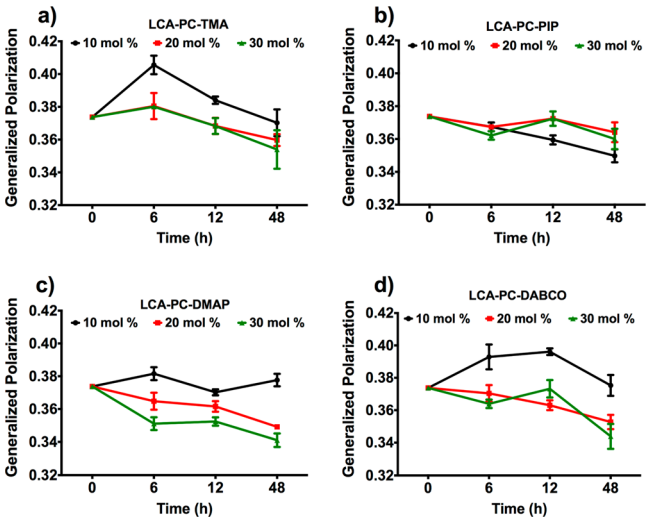

Figure 2. Change in generalized polarization of laurdan doped DPPC membranes on incubation with LCA phospholipids at different times intervals: (a) LCA-PC-TMA, (b) LCA−PC-PIP, (c) LCA-PC-DMAP, (d) LCA-PC-DABCO.

after 6 h, whereas incubation of corresponding cationic lipids induces dehydration of membranes due to strong electrostatic interactions with phospholipid membranes.[33] Therefore, LCA-PC-TMA cannot execute effective electrostatic interactions like cationic lipids with model membranes. In contrast, LCA-PC-PIP, LCA-PC-DMAP, and LCA-PC-DABCO in general induce a concentration dependent decrease in GP of laurdan suggesting the increase in hydration of DPPC membranes. Incubation of LCA-PC-DMAP is able to enhance the surface hydration of membranes even after 6 h (Figure 2). These studies suggest that hydration of model membranes induced by these phospholipids is strongly contingent on hydrophobicity of the headgroup. As a decrease in generalized polarization is indicative of enhanced membrane hydration, the order of induction of membrane hydration with LCA phospholipids on incubation of 30 mol % of phospholipids after 48 h is LCA-PC-DMAP > LCA-PC-DABCO > LCA-PC-PIP > LCA-PC-TMA.

Packing of DPPC lipids in model membranes is based on critical balance of strong hydrophobic interactions between acyl chains, and electrostatic repulsive forces between phospholipid charged headgroups. The enhanced hydration observed in DPPC membranes on incubation with LCA-PC-DMAP suggests that the incubation of this phospholipid enhances the repulsive forces between the charged headgroups of DPPC lipids. These enhanced repulsive forces therefore augment the hydration of membranes. A small headgroup based phospholipid like LCA-PC-TMA is not able to enhance these repulsive forces and therefore does not induce any change in hydration of membranes. Increase in hydrophobicity of charged headgroup disrupts this electrostatic balance of DPPC lipids in membranes and enhances its hydration.

Details for DPH based membrane rigidity studies follow. After initial interactions with membrane surface, phospholipids interact with hydrophobic acyl chains membranes for delivery to cytoplasm. Therefore, to find the changes in rigidity of membranes, we used diphenylhexatriene (DPH), a hydrophobic fluorophore that locates and orients in hydrophobic acyl chain regions of membranes. We incubated LCA phospholipids with DPH doped DPPC membranes and measured the change in steady state fluorescence anisotropy of DPH. Decrease in fluorescence anisotropy of DPH would be indicative of enhanced membrane fluidity.[23]

As shown in Figure 3, incubation of LCA-PC-TMA and LCA-PC-PIP does not induce much change in anisotropy of

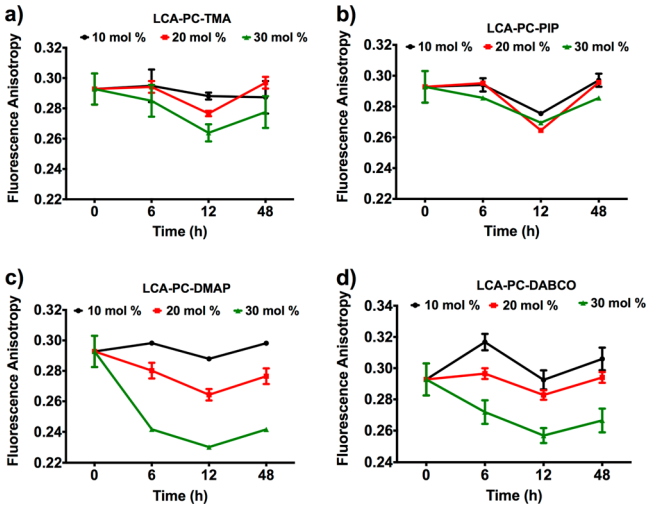

Figure 3. Change in fluorescence anisotropy of DPH doped DPPC membranes on incubation with LCA phospholipids: (a) LCA-PC-TMA, (b) LCA-PC-PIP, (c) LCA-PC-DMAP, (d) LCA-PC-DABCO.

DPH, keeping the membranes rigid in nature even after 24 h of incubation. Minimal change in anisotropy induced by LCA-PC-TMA and LCA-PC-PIP is also attributed to negligible change in membrane hydration as observed by laurdan studies. Therefore, LCA-PC-TMA and LCA-PC-PIP are not able to fuse with model membranes. Incubation of LCA-PC-DABCO lowers anisotropy of DPH, thereby suggesting the increase in

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fluidity of DPPC membranes. Interestingly, LCA-PC-DMAP induces maximum drop in anisotropy of DPH on its incubation suggesting the maximum fluidity of DPPC membranes on LCA-PC-DMAP incubation. Incubation of 30 mol % of LCA-PC-DMAP is able to induce the maximum membrane fluidity even at 6 h (Figure 3c). Therefore, these phospholipids follow the order of induction of membrane fluidity as LCA-PC-DMAP > LCA-PC-DABCO > LCA-PC-PIP ≈ LCA-PC-TMA which is incoherent with induction of membrane hydration observed during laurdan based hydration studies.

Hydrophobic regions of sterols usually disrupt the tight packing of phospholipid acyl chains and induce changes in membrane fluidity. In the case of LCA phospholipids, nature of the charged headgroup determines the fluidity of DPPC membranes in spite of the same hydrophobic sterol region. Aromatic charged headgroups induce maximum disruptions in DPPC membranes and increase its fluidity, whereas small charged trimethylammonium headgroups do not disrupt the membrane rigidity as they possess weak interactions with DPPC membranes. As bulky charged headgroups enhance membrane hydration, this enhanced hydration allows penetration of more water molecules and phospholipids inside the membranes. This enhanced permeation of phospholipids with bulky headgroups increases the fluidity of membranes. Our membrane fluidity studies are in agreement with laurdan studies suggesting that LCA-PC-DMAP molecules are more effective in interacting with model membranes, and therefore can be explored further for drug delivery applications.

Membrane Properties of LCA-PC Doped DPPC Membranes. Model phospholipid membranes doped with cholesterol, sterol phospholipids, and drugs have been explored for drug delivery applications.[12] Biophysical interactions of these doped drug molecules/lipids with model membranes are therefore critical to understand the drug encapsulation capacity and drug release properties of membranes.[37] We therefore studied the lipid−lipid interactions of DPPC membranes doped with LCA phospholipids by laurdan based hydration, DPH based membrane fluidity, and calorimetric experiments.

Details for membrane hydration studies follow. Laurdan doped DPPC-LCA-phospholipid membranes were studied to unravel the influence of doping of LCA phospholipids in membranes (Figure 4). Doping of LCA phospholipids except

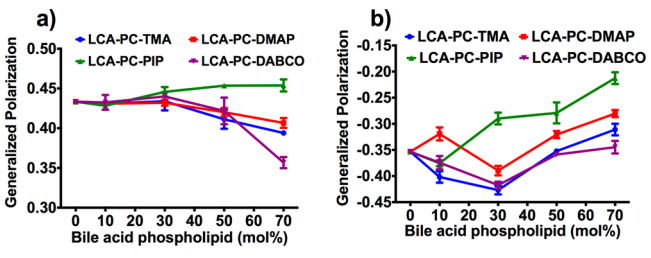

Figure 4. Change in general polarization (GP) of laurdan in (a) gel phase at 25 °C and (b) liquid crystalline phase at 55 °C of LCA phospholipid doped DPPC membranes.

LCA-PC-DABCO in DPPC membranes does not induce any significant change in hydration of membranes in gel phase (Figure 4a), whereas >30 mol % doping of LCA-PC-DABCO lowers the GP value of laurdan and enhances the membrane hydration by increased penetration of water molecules. The order of membrane hydration induced by the LCA phospholipids in gel phase is LCA-PC-DABCO > LCA-PC-TMA > LCA-PC-DMAP > LCA−PC-PIP (Figure 4a). This enhanced membrane hydration by LCA-PC-DABCO in gel phase of membranes might be due to ineffective packing of the bulky DABCO headgroups in DPPC membranes. This ineffective packing along with presence of extra tertiary amine in the case of LCA-PC-DABCO might allow more penetration of water molecules and enhance the hydration of membranes. In contrast, LCA-PC-TMA, LCA-PC-DMAP, and LCA−PC-PIP molecules are able to gel well with DPPC lipids, and may have effective interactions at the headgroup level with phosphocholine headgroups of DPPC lipids. In liquid crystalline (LC) phase (Figure 4b), doping of the LCA phospholipids at >30 mol % dehydrate the membrane surface, whereas at <30 mol % doping, we observed slight hydration of DPPC membranes. The order of membrane dehydration caused by LCA phospholipids in LC phase of membranes is LCA-PC-PIP > LCA-PC-DMAP > LCA-PC-TMA > LCA-PC-DABCO (Figure 4b) which is in contrast to the order of membrane hydration in gel phase by these phospholipids. These observations clearly suggested that LCA-PC-PIP possesses strong interactions with phosphocholine headgroups of DPPC lipids in gel phase of membranes, and therefore does not induce penetration of water molecules. Similarly, in LC phase, these strong interactions between LCA-PC-PIP and DPPC lipids induce membrane dehydration. In contrast, LCA-PC-DABCO with bulky headgroup could not hold these interactions effectively with phosphocholine headgroups of DPPC lipids. Therefore, LCA-PC-DABCO enhances the hydration of DPPC membranes in gel phase and induces minimum dehydration in LC phase of membranes.

Membranes in general make a transition from gel to liquid crystalline phase on increase in temperature. On increase in temperature, more water molecules penetrate in the membranes and enhance the membrane hydration. We studied the effect of temperature on hydration of LCA-phospholipid doped membranes. Increase in temperature in general lowers the GP of laurdan suggesting the greater hydration of DPPC membranes (Figure 5). Neat DPPC membranes show a sharp

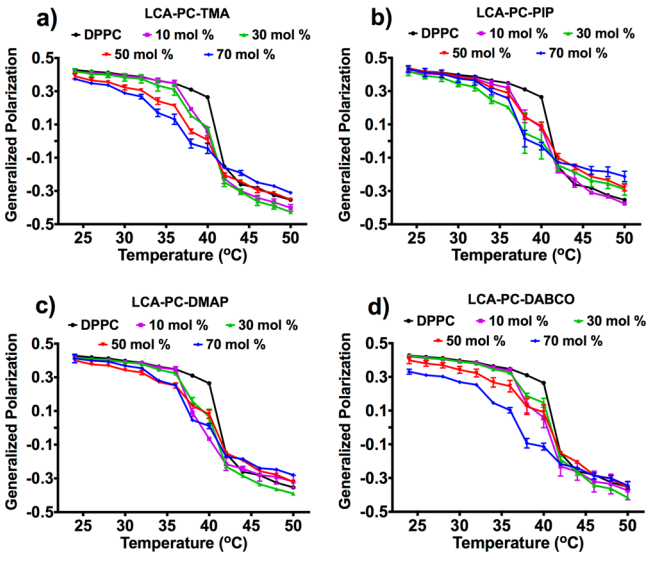

Figure 5. Change in generalized polarization (GP) of laurdan with increase in temperature of LCA phospholipid doped DPPC membranes: (a) LCA-PC-TMA, (b) LCA-PC-DMAP, (c) LCA−PC-PIP, (d) LCA-PC-DABCO.

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increase in hydration of membranes at phase transition temperature (T_m). Doping of LCA phospholipids disrupts this sharp transition in hydration of membranes depending on the nature of the charged headgroup. As doping of LCA-PC-DABCO enhances the hydration of gel phase due to bulky headgroup and imperfect packing, LCA-PC-DABCO doped DPPC membranes do not show a sharp change in hydration of membranes at T_m. All the phospholipids in general show change in membrane hydration over a broad range of temperature (Figure 5). Temperature based studies concluded that LCA phospholipids with different headgroups are able to interact with DPPC membranes effectively unlike cholesterol based lipids that abolish the DPPC membranes.[36] Induction of hydration in DPPC membranes is contingent on hydrophobicity of the headgroup as bulky headgroups induce more loose packing within membranes and enhance the membrane hydration.

We then explored the effect of doping of LCA phospholipids on membrane fluidity using DPH based anisotropy experiments. These studies would help us to find the thermostability of ordered domains in DPPC membranes, where DPH partitions equally between disordered and ordered phases. The presence of an ordered domain would protect a fraction of DPH and recognize its quenching susceptibility that would allow us to know about the thermostability of ordered domains as a function of temperature.[39]

Doping of all the phospholipids induces concentration dependent lowering of DPH anisotropy in gel phase of DPPC membranes, implying that addition of phospholipids induces membrane fluidity in gel phase (Figure 6a).

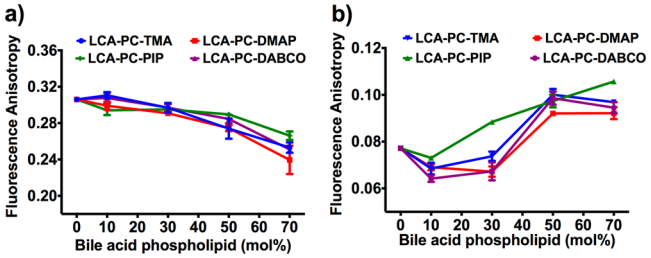

Figure 6. Change in fluorescence anisotropy of DPH in (a) gel phase at 25 °C and (b) liquid crystalline phase at 55 °C of LCA phospholipid doped DPPC membranes.

Introduction of different headgroups with varying hydrophobicity and steroidal backbone of phospholipids might have created packing defects in DPPC membranes to enhance its membrane fluidity. In liquid crystalline (LC) phase, doping of LCA phospholipids induces concentration dependent rigidification of DPPC membranes implying that LCA phospholipids induced liquid ordered domains in the DPPC membranes (Figure 6b).

DPPC membranes in gel phase are tightly packed due to strong hydrophobic van der Waals interactions between long acyl chains. These hydrophobic acyl chains are randomly oriented in liquid crystalline phase at high temperature. Increase in temperature brings the transition from gel to liquid crystalline phase by inducing the random motions in acyl chains of DPPC membranes. We therefore studied the effect of temperature on motion of these acyl chains in LCA-phospholipid doped DPPC membranes by measuring the change in anisotropy of DPH in membranes. Neat DPPC membranes showed a sharp decrease in anisotropy of DPH at phase transition temperature suggesting the fluidic nature of membranes at high temperature. As doping of LCA phospholipids enhances the fluidity of membranes in gel phase, we did not observe this sharp change in membrane fluidity transition on increase in temperature in LCA phospholipid doped membranes (Figure 7). In general, LCA

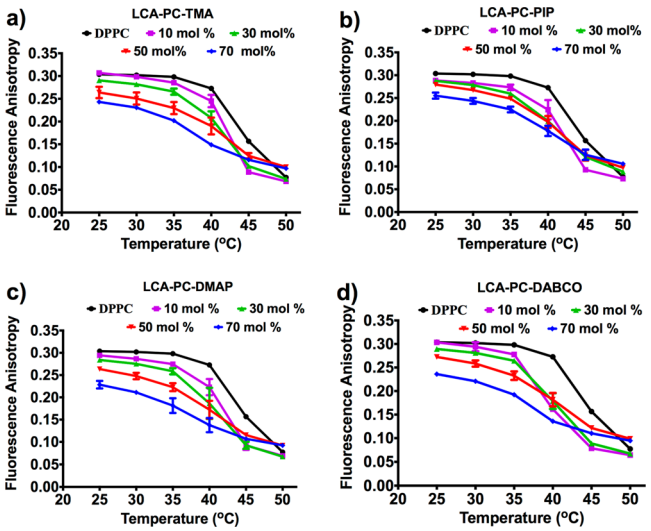

Figure 7. Change in fluorescence anisotropy of DPH with increase in temperature of LCA phospholipid doped DPPC membranes: (a) LCA-PC-TMA, (b) LCA-PC-PIP, (c) LCA-PC-DMAP, (d) LCA-PC-DABCO.

phospholipid doped DPPC membranes execute a change in membrane fluidity over a broad range of temperature, suggesting the noncooperative interactions between LCA phospholipid and DPPC acyl chains. We have not observed any abolition of phase transition even at higher doping concentrations (70 mol %) in contrast to cholesterol doped membranes as they abolish phase transition even at >30 mol % doping.[38] LCA-PC-DMAP and LCA-PC-DABCO with bulky headgroups induce maximum broadness in phase transition of membranes as compared to LCA-PC-TMA and LCA-PC-PIP (Figure 7). Therefore, interactions of different headgroups with varying hydrophobicity at the membrane surface level influences the rigidity of DPPC membranes. Absence of abolition of phase transition on doping of LCA phospholipids suggests that these amphiphiles have better packing capability as compared to cholesterol DPPC membranes, and bulky polar headgroups help in incorporation of these phospholipids in DPPC membranes. As membrane fluidity is strongly contingent on the physicochemical properties of the headgroups and percentage of doping, these conjugates can be useful candidates for drug delivery.

To further delineate the packing of phospholipids in DPPC membranes, we analyzed these coliposomes with high sensitive differential scanning calorimetry. Pure DPPC liposomes show a pretransition at 35.7 °C followed by a main transition at 41.3 °C. Pretransition peak of DPPC membranes usually gets abolished on doping of LCA phospholipids. LCA-PC-TMA on 30 mol % doping broadens the main phase transition (Figure 8), that gets further broadened on increase in doping (50 and 70 mol %) of LCA-PC-TMA in DPPC membranes without change in T_m. Similarly, doping of LCA-PC-PIP, LCA-PC-DMAP, and LCA-PC-DABCO broadens phase transition of

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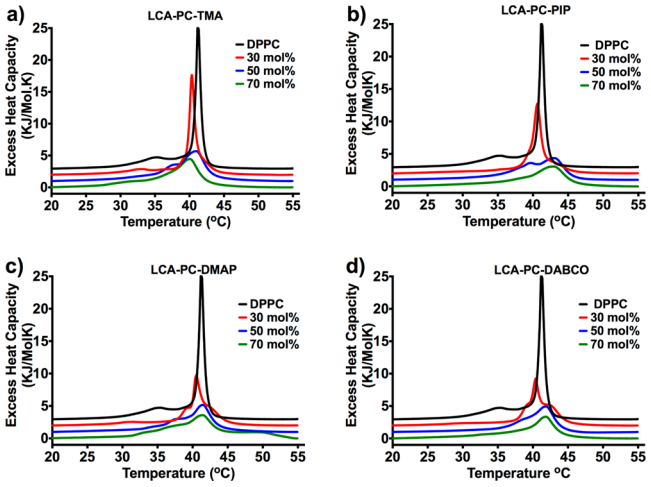

Figure 8. Differential scanning calorimetry thermograms of LCA phospholipid doped DPPC membranes: (a) LCA-PC-TMA, (b) LCA-PC-PIP, (c) LCA-PC-DMAP, (d) LCA-PC-DABCO. Thermograms have been successively lowered for clarity.

DPPC membranes even at 30 mol % doping (Figure 8). Doping with higher concentrations (up to 70 mol %) of these phospholipids does not lower the T_m of membranes, suggesting the van der Waals interaction between acyl chains of DPPC and bile acid backbone. DSC endotherms of liposomes on doping with high mol % of LCA phospholipids are the sum of two superimposed sharp and broad components indicating the existence of different lipid rich domains (Figure 8).[23] These phospholipids can easily integrate in DPPC membranes through interactions at headgroup level and van der Waals associations between sterol backbone and acyl chains in the hydrophobic domains. Doping of >50 mol % of LCA-phospholipids broadens the transition and forms different domains, suggesting the formation of DPPC rich and LCA phospholipid rich domains.

We then computed the thermodynamic parameters such as calorimetric enthalpy (ΔH_c), van't Hoff enthalpy (ΔH_{vH}), and cooperativity of transition (Table 1). A complex trend of calorimetric enthalpies was observed on doping of LCA phospholipids in DPPC membranes. Doping of these phospholipids in DPPC membrane increases the FWMH and reduces the height (C_p^{max}) of transition peak. LCA phospholipids in general show reduction in van't Hoff enthalpy with increasing doping concentration. Van't Hoff enthalpies in the case of lipid bilayer are larger than calorimetric enthalpies because of intermolecular cooperation. Van't Hoff enthalpies and cooperativity unit decrease with increase in doping of phospholipids (Table 1).

Calorimetry studies in combination with laurdan based hydration and DPH based anisotropy studies also depict that headgroups of LCA phospholipids perform differential interactions with the phosphocholine headgroups of DPPC membranes. LCA-PC-TMA and LCA-PC-PIP lipid molecules might perform electrostatic interactions with phosphocholine headgroups through mutual cationic and phosphate charged groups. LCA-PC-DMAP amphiphile might be performing cationic charge-π bridge interactions between phosphocholine headgroup and aromatic dimethylamino headgroup, whereas the introduction of bulky DABCO headgroup performs weak interactions. These interactions followed by hydrophobic interactions between the sterol part of lithocholic acid and acyl chains of DPPC phospholipids help in high retention of LCA phospholipids in membranes, whereas DPPC is not able to hold such high cholesterol content and induce its separation of membranes.[40] Recently Szoka and co-workers have reported the membrane properties of cholesterol based phospholipids and showed 60 mol % incorporation of these cholesterol phospholipids in model membranes.[10] Therefore, these LCA based phospholipids are able to form stable vesicular suspensions even upon incorporation of 70 mol % bile acid phospholipids, and no precipitation was observed on storage at 4 °C. Additionally absence of increased scattering from these coliposomal membranes confirms any exclusion of phospholipids from membranes.

CONCLUSIONS

Headgroup chemistry of phospholipids plays a critical role in fusion of drug delivery carriers with target cell membranes. We varied different polar headgroups on lithocholic acid phospholipids from small hydrophilic to large hydrophobic headgroups, and studied their interactions with model membranes. Our studies showed that introduction of hydrophobic headgroup on phospholipids enhances the hydration and fluidity of membranes. From incubation studies, we

Table 1. Thermodynamic Characterization of DPPC and Its Coliposomes with Lithocholic Acid-Phospholipids Determined by Differential Scanning Calorimetry

amphiphile	doping (mol %)	T_m (°C)	ΔH_C (kcal/mol K)	fwhm[a]	C_p^{max}[b] (kcal/mol K)	ΔH_{VH} (kcal/mol K)	CU[c]
DPPC		41.30	9.29	0.97	4.84		
LCA-PC-TMA	30	40.43	6.61	1.22	1.14	93.84	14.19
	50	41.90	5.23	5.73	0.79	59.80	11.44
	70	41.88	5.22	5.16	0.62	28.13	5.39
LCA-PC-PIP	30	40.68	7.06	1.86	2.07	160.65	22.75
	50	43.16	6.52	7.31	0.69	41.83	6.41
	70	42.98	5.77	6.00	0.62	25.47	4.42
LCA-PC-DMAP	30	40.61	6.59	3.54	1.21	100.26	15.21
	50	41.48	6.17	6.04	0.74	47.30	7.67
	70	41.43	8.05	9.13	0.60	17.54	2.18
LCA-PC-DABCO	30	40.36	7.56	4.16	3.12	225.51	29.82
	50	41.01	7.43	5.12	0.92	48.60	6.54
	70	40.13	7.32	4.87	0.85	27.10	3.70

[a]Full width at half-maximum. [b]Maximum point of specific heat capacity. [c]Size of cooperativity unit.

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conclude that LCA-PC-DMAP is more potent in inducing membrane hydration and enhancing the membrane fluidiy. Interestingly, hydration and fluidity of LCA phospholipid doped DPPC membranes is contingent on nature of the charged headgroup on LCA phospholipids. Calorimetric studies showed that doping of these phospholipids did not cause phase transition of DPPC lipids to be abolished. Our findings would further help in designing new steroidal phospholipids with unnatural headgroups that would fuse with target biomembranes effectively for drug delivery.

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Notes

The authors declare no competing financial interest.

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