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Interaction of choline salts with artificial biological membranes: DSC studies elucidating cellular interactions☆

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ABSTRACT

To better understand the relationship between the relative cytotoxicity of diluted ionic liquids and their specific interaction with biological membranes, the thermotropic behavior of model lipid membrane systems formulated in a series of choline based organic salts was investigated. Unilamellar vesicles prepared from dipalmitoylphosphatidylcholine were exposed to a series of choline phosphate salts at a concentration of 10 mM at pH 7.40, and the gel to liquid-crystalline state transition was examined using differential scanning calorimetry. The choline salts that were observed to have a low relative toxicity in previous studies induced minimal changes in the lipid phase transition behavior of these model membranes. In contrast, the salts choline bis(2,4,4-trimethylpentyl)phosphinate and choline bis(2-ethylhexyl)phosphate, both of which were observed to have high relative toxicity, caused distinct disruptions in the lipid phase transition behavior, consistent with penetration of the salts into the acyl chains of the phospholipids. choline bis(2,4,4-trimethylpentyl)phosphinate reduced the T_m and enthalpy of the main transition of dipalmitoylphosphatidylcholine while choline bis(2-ethylhexyl)phosphate induced the equilibration of alternate phases.

1. Introduction

Low melting point organic salts (T_m < 100 °C), frequently referred to as ionic liquids (ILs), have recently been explored as solvents/co-solvents for a variety of biotechnology applications, ranging from the formulation of biopharmaceuticals to media for biocatalysis [1–3]. In some cases these ILs are being studied as pure solvents, with little to no water present, and in other applications they are studied in composition with water, sometimes with water as the dominant co-solvent [1,2,4,5]. The growing potential for ILs in this arena raises questions as to how ILs behave in the human body and/or in ecosystems. The disposition of ILs in the environment or in the human body will be largely dictated by the behavior of the organic ions after they have been diluted into a predominantly aqueous environment. To date, biological compatibility of ILs formulated in aqueous media has generally been explored in the context of high-throughput assays which study the relative toxicity of ILs towards particular species or cell types [3,6–9]. While these types of assays provide useful information as to how certain chemical structures affect toxicity trends, the information does not elucidate the specific molecular interaction with cellular organelles, such as the cell membrane.

Much of what we currently know about the nature of IL interactions with membranes originates from observations made from cytotoxicity studies. Alkylimidazolium salts have been most extensively tested, revealing a direct link between alkyl side-chain length and cell toxicity. It has been suggested that toxicity is linked to lipophilicity, where hydrophobic side chains increase membrane permeability and thus cause necrotic effects [8,10,11]. In previous cytotoxicity studies with choline based ILs, our group observed that as alkyl chain length and branching increased on the anionic component of the IL, the cytotoxicity to mammalian cells increased [3]. This increase in cytotoxicity was hypothesized to be related to an increase in lipophilicity, and thus more disruptive interactions with mammalian cell membranes. It has also been shown by others that the adverse effects of anionic surfactants on viability and growth of different marine microalgae species appear to be related to interaction between the outer plasmatic-membrane of the cell with compounds available in the environment [12]. Both electrostatic interactions at the membrane surface and hydrophobic interactions with lipid acyl chains can influence the penetration of hydrophobic compounds into the core of membranes by altering lipid arrangement. The nature of these interactions can cause physical perturbations of the membrane that lead to cell leakiness, and penetration of compounds across the membrane, thus enabling interactions with intra-cytoplasmic organelles.

Abbreviations: CBEH, choline bis(2-ethylhexyl)phosphate; CDBP, choline dibutyl phosphate; CDEP, choline *O,O′*-diethyl dithiophosphate; CDHP, choline dihydrogen phosphate; CTMP, choline bis(2,4,4-trimethylpentyl)phosphinate; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; DSC, differential scanning calorimetry; IL, ionic liquid

☆ Electronic supplementary information (ESI) available: chemical structures of compounds investigated.

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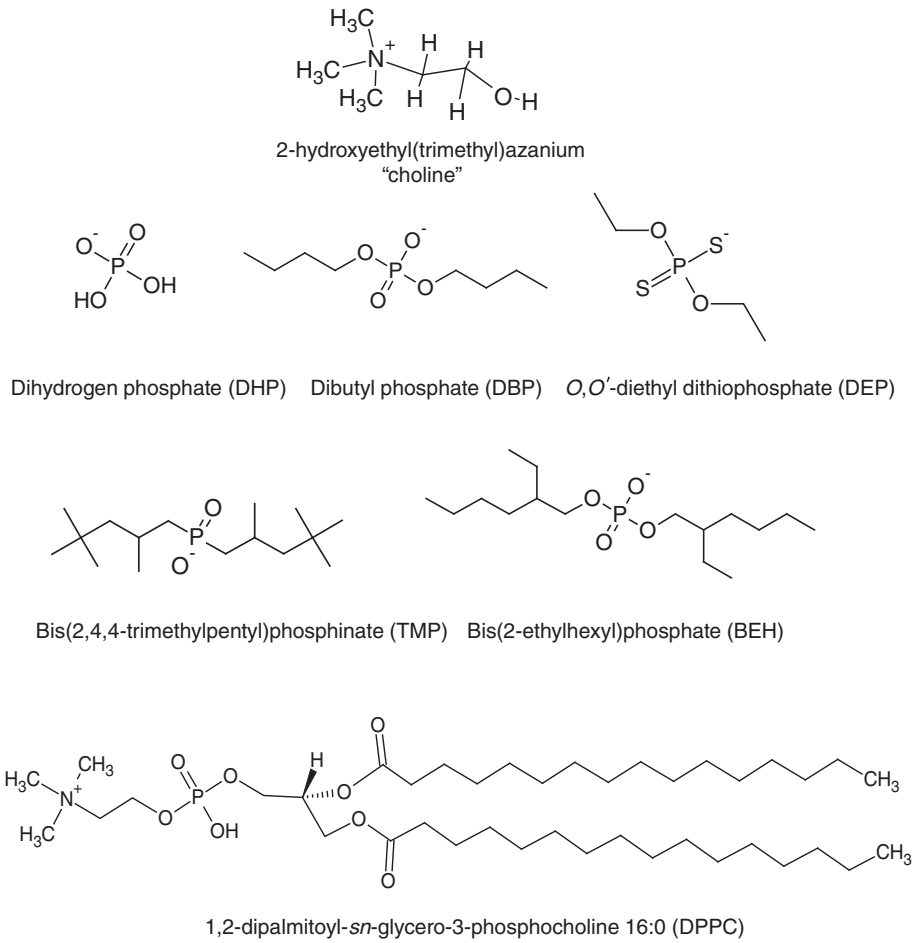

Fig. 1. Chemical structures of the ion components of the choline salts: choline dihydrogen phosphate (CDHP), choline dibutyl phosphate (CDBP), choline *O,O′*-diethyl dithiophosphate (CDEP), choline bis(2,4,4-trimethylpentyl) phosphinate (CTMP), choline bis(2-ethylhexyl)phosphate (CBEH), and 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC).

To better understand the nature of specific interactions between ILs and biological interfaces, we have investigated in this work the effect of certain choline based ILs and organic salts on the thermotropic phase behavior of model lipid membrane systems prepared from 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) (Fig. 1). Using calorimetric methods we examined the heat signals associated with the lamellar gel (L_β) to lamellar liquid crystalline (L_α) phase transition of unilamellar vesicles prepared from DPPC. The temperature at which this event occurs can be described as the melting temperature (T_m) of the hydrocarbon chains. Because of the tight packing and restricted mobility of hydrocarbon acyl chains in a bilayer arrangement, this transition from the all-trans conformation to the gauche conformation is a highly cooperative event [13]. Molecules that interact or intercalate with the acyl chains can significantly affect the cooperativity of this transition. Thermal parameters that were monitored to probe bilayer effects include the temperature at the transition peak (T_m), the width of the transition peak at half height ($\Delta T_{1/2}$), and the enthalpy of the transition change (ΔH) [14].

2. Materials and methods

2.1. Materials

Lyophilized lipid 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine 16:0 (DPPC) was purchased from Avanti Polar Lipids (Alabaster, AL). Sodium chloride was obtained from Fisher Scientific (Pittsburgh, PA). Crystalline sodium phosphate dibasic, 7-hydrate ($Na_2HPO_4 \cdot 7H_2O$) was purchased from J. T. Baker (Phillipsburg, NJ). Bis(2,4,4-trimethylpentyl) phosphinic acid was obtained from Cytec Industries (Woodland Park, NJ). Choline chloride, choline hydroxide (20 wt.% in H_2O), methanol, dibutyl phosphate, bis(2-ethylhexyl) phosphate, *O,O′*-diethyl dithiophosphate, nortriptyline hydrochloride, procainamide hydrochloride, ibuprofen sodium salt, and 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (HEPES) were all purchased from Sigma Aldrich (St. Louis, MO). All chemicals were used as received, except as otherwise specified. ELGA Purelab Ultra water (18.2 MΩ.cm) was used in the preparation of all water based solutions.

2.2. Synthesis of choline salts

Five choline salts were prepared at Monash University according to previous methods:

(1) 2-hydroxyethyl(trimethyl)azanium dihydrogen phosphate (CDHP, choline dihydrogen phosphate), melting point = 195 °C [15],[1]
(2) 2-hydroxyethyl(trimethyl)azanium bis(2,4,4-trimethylpentyl) phosphinate (CTMP, choline bis(2,4,4-trimethylpentyl)phosphinate), melting point = 131.2 °C [3],
(3) 2-hydroxyethyl(trimethyl)azanium *O,O′*-diethyl dithiophosphate (CDEP, choline *O,O′*-diethyl dithiophosphate), melting point = −43.6 °C [3],
(4) 2-hydroxyethyl(trimethyl)azanium dibutyl phosphate (CDBP, choline dibutyl phosphate), melting point = 79 °C [3,16]

[1] Correction of previously reported T_m = 119 °C. [4] K. Fujita, D.R. MacFarlane, M. Forsyth, Protein solubilising and stabilising ionic liquids, Chemical communications (Cambridge, England), (2005) 4804–4806.

(5) 2-hydroxyethyl(trimethyl)azanium bis(2-ethylhexyl)phosphate (CBEH, choline bis(2-ethylhexyl)phosphate). Melting point is not well defined, but there is a glass transition (onset temperature) = −83.2 °C [3].

Melting points were determined using a TA Instruments Q100 differential scanning calorimeter (DSC) over a temperature range of −100 to 200 °C at a scanning rate of 10 °C/min. CDHP and CTMP are solids at room temperature, but with the addition of small amounts of water [≤20% (w/w)] produce clear liquids. However, they are considered as near-ILs since they are liquid at room temperature when hydrated with 20% (w/w) H_2O [17]. CDBP is a liquidous salt (semi-crystalline) at room temperature. CDEP and CBEH are viscous liquids at room temperature.

2.3. Preparation of unilamellar vesicles

To study model membranes, a mini-extruder was used as described in the Avanti Polar Lipids, Inc. protocol for preparing large, unilamellar vesicles by extrusion as found at http://Avantilipids.com. In order to prepare unilamellar liposomal suspensions with a low polydispersity, membranes having a pore size of ≤0.2 μm were used as recommended. Stock lyophilized DPPC was stored under an argon atmosphere in a −20 °C freezer until used. To prepare lipid solutions, lyophilized DPPC powder was placed into disposable glass culture tubes with two glass beads and 20 mM HEPES buffer at pH 7.40, which had been pre-warmed to 70 °C using a isotemp heating block (Fisher Scientific, Pittsburgh, PA). The lipid solutions were then vortexed, and placed in the heating block at 70 °C for 30 min with occasional agitation to allow complete hydration. The lipid solutions were then extruded through Nucleopore® track-etched polycarbonate filters (pore size 100 nm) (Whatman, Pleasanton, CA) mounted in a mini-extruder (Avanti Polar Lipids, Alabaster, AL) fitted with two 1.0 mL Hamilton gas-tight syringes (Avanti Polar Lipids, Alabaster, AL). In order to achieve a tighter polydispersity (particle size distribution), the lipid solutions were subjected to 21 passes through the two filters after which the lipid vesicle solutions were collected from the receiving syringe to prevent contamination by particles that had not passed through the filter. The extrusions were refrigerated at 4 °C for a minimum of 16 h before use in calorimetry experiments.

2.4. Differential scanning calorimetry (DSC)

A MicroCal VP-DSC microcalorimeter (MicroCal, Northampton, MA) was used to assess the thermal stability of all samples. DSC baseline repeatability was established with a minimum of 5 reference solution scans. The DPPC dispersions were prepared and allowed to equilibrate overnight (16 h) at 4–5 °C. The dispersions were not incubated longer than overnight in order to limit the inducement of the subgel phase which normally occurs within 4–5 days at 0–4 °C [18]. After the refrigerated incubation period, and just prior to loading samples into the DSC, the lipid dispersions were gently allowed to warm to room temperature. An aliquot of the stock lipid vesicle solution was combined with experimental compounds in 20 mM HEPES buffer at pH 7.40 in a 1:1 ratio by volume where dilution resulted in a 10 mM test compound experimental concentration in the calorimeter sample cell. The experimental test compound concentration of 10 mM was chosen to allow a comparison of membrane interactions at a level where toxic and non-toxic interactions could be discriminated [3]. Enough stock lipid vesicle solution was prepared initially to allow a control (lipid only) DSC run for each compound tested. The test solutions were mixed in thermovac tubes (MicroCal, Northampton, MA), and immediately degassed for 5 min using a thermovac vacuum degassing system (MicroCal, Northampton, MA). After degassing, the samples were loaded by syringe into the calorimeter. After loading into the calorimeter sample cell, the samples were allowed to equilibrate 15 min at the initial starting temperature of either 10 or 20 °C then scanned from 20 to 50 °C or from 10 to 90 °C at 60 °C/h with a 16 second filter and passive feedback (gain). Reversibility was determined by rescanning samples after allowing them to cool down to initial temperature. The samples were repeatedly scanned until at least three consistent thermograms were obtained consecutively. The data was analyzed using Origin software provided by MicroCal in order to permit the determination of the main transition temperature (T_m), the main transition full-width-at-half-maximum-height ($\Delta T_{1/2}$), and the calorimetric enthalpy (ΔH).

3. Results and discussion

In previous work we have shown that for a series of choline salts significant differences were observed in the biocompatibility of these salts with a macrophage cell line over a 48 hour exposure period. Mouse macrophage (J774) cells were exposed to choline salts that were added to the growth medium which consisted of Dulbecco's Modified Eagle's Medium (DMEM) with inorganic and organic components at original concentrations and supplemented with 4500 mg/L D-glucose and 110 mg/L sodium pyruvate [3]. These chronic exposure studies enabled a rank ordering of salts based on the concentration required to reduce survival to 50% after 48 h of exposure (EC_{50}). Some of the choline salts were as benign as typical physiologic salts, causing injury only when the concentration reached a level that would induce osmotic stress (CDHP, CDBP, and CDEP). Other choline salts (CTMP and CBEH) reduced survival at much lower concentrations. The apparent cellular toxicity appeared to trend with the lipophilicity of the compound, and we hypothesized that adverse interactions with the lipid cell membrane were the source of this toxicity [3]. The mammalian cell plasma membrane consists of a variety of biomacromolecules, arranged into complex assemblies that are not readily amenable to calorimetric study. The primary components are proteins and lipids, and simple, single-component bilayer lipid vesicles are often used as model systems for cells [19–21]. To better understand the nature of lipid membrane interactions, model vesicles formed from phospholipids were exposed to choline salts and other well-characterized control compounds to elucidate perturbing effects on the model membranes that might lead to leakiness or general loss of membrane structure. DPPC was chosen as the vesicle forming lipid due to the fact that the major fatty acid in the choline-containing class of glycerophospholipids found in mouse macrophages is palmitic acid (16:0) [22]. The DPPC model membrane being used in this DSC study is thus intended to serve as the most basic mimic of the mouse macrophage cell membrane.

In order to identify the specific thermal signatures associated with different types of interfacial interactions, control compounds with well-known effects on cell membranes were added to DPPC vesicles, and changes in the membrane transition behavior of the lipids were evaluated using DSC (see supplementary information Fig. S1 for structures of control compounds). DSC measures enthalpy changes associated with a given phase change, and the endotherms for DPPC vesicles in 20 mM HEPES at pH 7.40 without added control compounds exhibited the characteristic thermotropic behavior, demonstrating both a pretransition at 36 °C and a gel–liquid crystal phase transition at 41.4 °C, in agreement with previous reports (Fig. 2A) [23]. Mixtures of choline salts and DPPC vesicles were studied at similar lipid concentrations (approximately 0.39 mM) under the same experimental conditions to allow comparison of specific interactions between the salts and the lipid vesicles. Simple physiological salts were also included for comparison. In order to compare the thermotropic behavior of DPPC in relation to each compound, the main transition temperature, the enthalpy change, and the level of cooperativity ($\Delta T_{1/2}$) with respect to each compound were gathered for comparison in Table 1, along with indications regarding pretransition effects. Previously published EC_{50} (mM) values for the set of choline salts

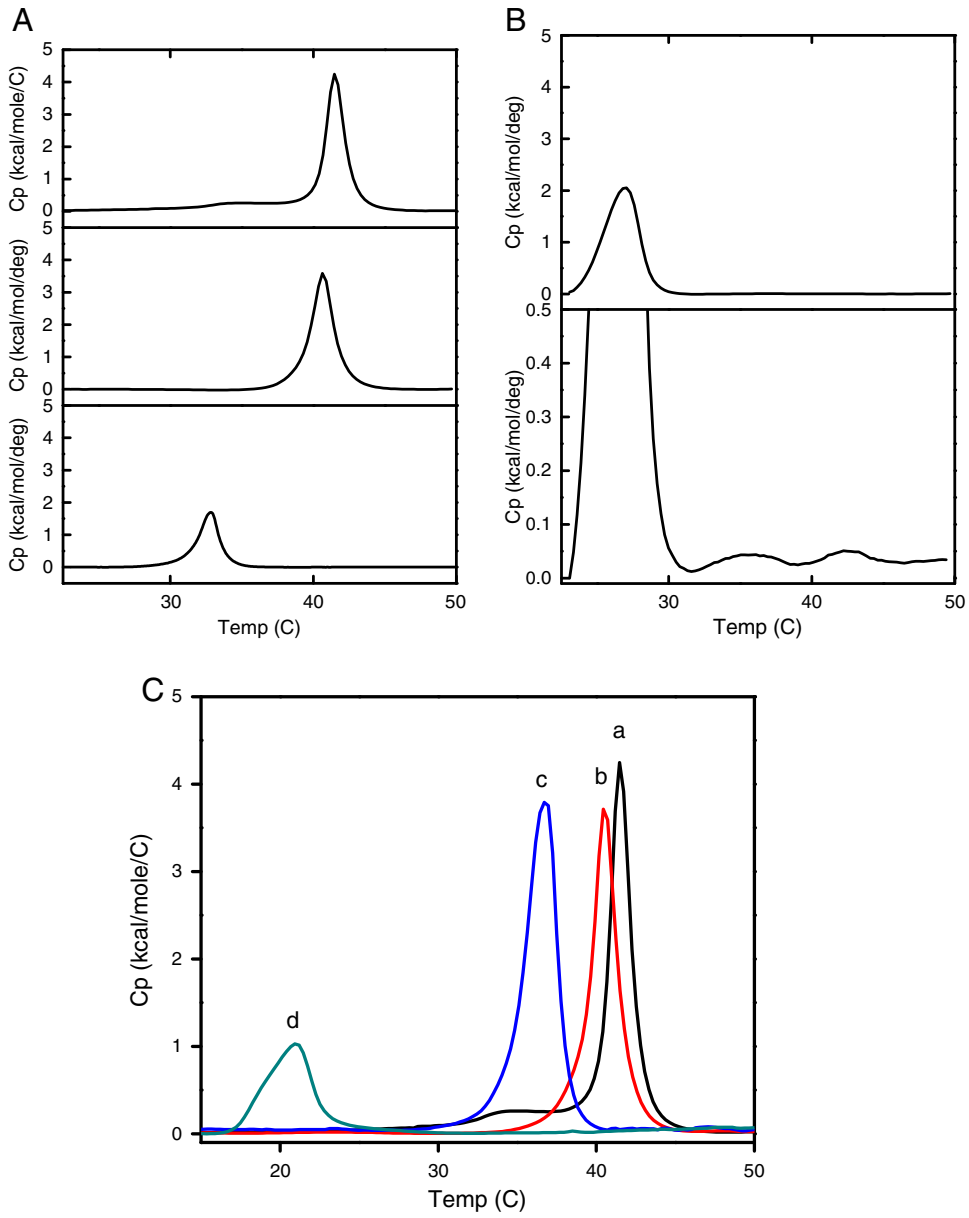

Fig. 2. Calorimetric curves for lipid bilayer mixtures. All solutions were prepared at pH = 7.40 in 20 mM HEPES. Panel A: Representative calorimetric curves for DPPC control in 20 mM HEPES (top), 10 mM CDEP (center) and 10 mM CTMP (bottom) lipid bilayer mixtures. Panel B: Representative calorimetric first scan curves for 10 mM CBEH lipid bilayer mixtures showing a dominate transition at 27.0 °C (top) and reduced main transitions (bottom). Panel C: DSC thermograms of DPPC liposomes in (a) in 20 mM HEPES main transition T_m = 41.1 °C, (b) 0.1 mM CBEH, second scan, T_m = 40.6 °C, (c) 1.0 mM CBEH, second scan, main transition T_m = 36.4 °C, and (d) 10 mM CBEH, second scan, subgel transition T_m = 20.0 °C.

investigated here are also reproduced in Table 1 where the compounds are ranked based on their relative cellular toxicity. Initial heating scans were also compared with subsequent reheating scans of the same sample.

3.1. Low toxicity salts

As shown in Table 1, CDHP, and CDBP had no statistically significant effect on the DPPC main gel to liquid-crystal phase transition (ΔT_m, $\Delta\Delta H$) and minimal effect on the change in the width of the transition at half-height ($\Delta\Delta T_{1/2}$) suggesting that there was no interaction between these salts and the acyl chains of the lipids at the studied concentration. The most benign salts tested previously for cytotoxicity were NaCl and choline chloride (CCl) with EC_{50s} of 63 and 34 mM respectively (Table 1) [3]. The thermotropic behavior of these two salts was comparable to the low toxicity choline compounds, as well as the control compound procainamide, all having little to no effect on the main transition enthalpy and little effect on T_m and cooperativity ($\Delta\Delta T_{1/2}$) compared to control DPPC runs from the same lipid batch solutions (Table 1). Procainamide has previously been classified as nonmembrane active towards dimyristoylphosphatidylcholine (DMPC), and appears to have the same effect on DPPC [21]. This suggests that the variation in toxicity observed with these compounds (CDHP: EC_{50} = 20 mM) and (CDBP: EC_{50} = 9 mM) when compared to more cellularly benign NaCl (EC_{50} = 60 mM) might have more to do with an aspect of cellular function, such as alteration of membrane proteins, ionic strength effects, nutrient sequestration or metal chelation, and does not appear to be due to lipid membrane localization (Table 1).

It was noted that the pretransition peak normally seen at approximately 36 °C was not resolvable in the thermal scans of DPPC in the presence of CDEP (EC_{50} = 8 mM) while the main transition

Table 1
Thermodynamic parameters of the phase transitions undergone by 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine 16:0 PC (DPPC) in different liposome preparations.[a]

Sample[b,c]	Main transition			Change in main transition[d]			Pretransition	Change in pretransition[d]	EC_{50}[e] (mM)
	T_m (°C)	ΔH (cal mol^{-1})	$\Delta T_{(1/2)}$ (°C)	ΔT_m (°C)	ΔΔH (cal mol^{-1})	$\Delta\Delta T_{(1/2)}$ (°C)	ΔH (cal mol^{-1})	ΔH (cal mol^{-1})	
Control compounds									
Nortriptyline hydrochloride	30.66 (0.05)	8078 (78)	1.41 (0.04)	−26%	+8%	+6%	NR	−100%	–
Procainamide hydrochloride	41.46 (0.01)	7892 (209)	1.44 (0.03)	+0.2%	+0.3%	−9%	173 (13)	−47%	–
Ibuprofen sodium salt	38.82 (0.15)	7222 (170)	1.79 (0.04)	−6%	−11%	+12%	375 (47)	−13%	–
Choline salts									
CDHP	41.69 (0.01)	7949 (121)	1.33 (0.00)	+0.7%	+3%	−6%	248 (14)	−54%	20
CDBP	41.05 (0.13)	7831 (218)	1.50 (0.09)	−0.5%	+2%	−6%	440 (73)	−20%	9.1
CDEP	40.68 (0.02)	8102 (53)	1.71 (0.04)	−2%	+2%	+14%	NR	−100%	8.2
CBEH [f]	42.49 (1.21)	77 (>23)	2.95 (>0.90)	+2.5%	−99%	+16%	66 (>20)	−82%	<0.30
CTMP [g]	32.58 (0.09)	3580 (11)	1.48 (0.00)	−21%	−52%	−17%	NR	−100%	<0.25
Inorganic/high melting point organic salts									
NaCl	41.72 (0.03)	9823 (134)	1.53 (0.06)	+0.8%	+17%	−4%	508 (38)	+103%	63
CCl	41.70 (0.02)	9633 (133)	1.55 (0.04)	+0.8%	+15%	−3%	378 (7)	+51%	34

[a] All samples were prepared in 20 mM HEPES buffer at pH 7.40. All compound concentrations are 10 mM. A control DPPC scan was obtained on the stock LUVs batch solutions as prepared for all compounds tested. Triplicate testing was run for all compounds except where otherwise noted. Values reported are for equilibrium scans which were reproducible after rescanning for a minimum of four scans unless otherwise noted. Not resolvable (NR) under these experimental conditions. The standard error is given in parenthesis.
[b] CDHP, choline dihydrogen phosphate; CTMP, choline bis(2,4,4-trimethylpentyl)phosphinate; CDEP, choline *O,O′*-diethyl dithiophosphate; CDBP, choline dibutyl phosphate; CBEH, choline bis(2-ethylhexyl)phosphate; CCl, choline chloride; NaCl, sodium chloride.
[c] Grand average from all DPPC control runs combined: Main Transition $T_m = 41.35 \pm 0.05$ °C, $\Delta H = 7892 \pm 100$ cal mol^{-1}, $\Delta T_{1/2} = 1.62 \pm 0.06$ °C; Pretransition $\Delta H = 364 \pm 26$ cal mol^{-1}, $n = 14$.
[d] Change in thermodynamic parameters induced by compound compared to DPPC control scan from same LUV batch solution.
[e] EC_{50} values reproduced from Weaver et al. [3].
[f] The gel-to-liquid-crystalline transition is not reversible in the presence of 10 mM CBEH, i.e., there is not an observable main transition after cooling and rescanning in the presence of this compound. The resolution of the pretransition and main transition peaks is low, and there is a higher degree of error in the calculated enthalpies.
[g] Average values from two replicates.

demonstrated significant broadening indicating a less cooperative gel to liquid-crystal phase transition (Fig. 2A, Table 1). This might suggest that CDEP is interacting with the surface of the lipid bilayer in a manner similar to that of nortriptyline. With the addition of nortriptyline to DPPC, the thermal transitions were shifted to lower temperatures, with notable main peak broadening ($\Delta\Delta T_{1/2}$) indicating reduction in the molecular ordering of lipid molecules within the vesicle bilayer and reduction in the cooperativity of melting without altering the overall domain organization to such an extent that overall enthalpy was changed [20,21]. A previous confocal Raman spectroscopy investigation on the partitioning of nortriptyline into DPPC vesicles indicated that this molecule is located in the lipid interfacial/headgroup region with the cyclic rings of the structure associating near the acyl chains with the polar carbon tail "snorkeling" into the lipid headgroup [20,21]. Head group interactions of this nature can cause a significant depression in the transition temperature of the gel to liquid-crystalline phase change [20]. In the case of CDEP this effect appears to be moderate, but could account for the lower gel to liquid-crystal phase transition and the slightly lower EC_{50} value compared to CDHP and CDBP.

3.2. High toxicity salts

The organic salts CBEH and CTMP were both observed to have a high toxicity in previous studies. Their structures suggest that they would be highly lipophilic, and thus likely to cause perturbations in membrane structure that would affect lipid phase transitions. Changes in lipid transition behavior were indeed observed, and indicate a certain level of lipid interaction. The salt CTMP (Log EC_{50} = <2.39 μM) reduced the main transition T_m to 32.6 °C compared to that for DPPC alone, reduced the transition enthalpy by half, and increased the cooperativity of the transition, as measured by a decrease in the peak width ($\Delta\Delta T_{1/2}$) by 17%, compared to control lipid vesicles run from the same stock batch solution (Fig. 2A, Table 1). CTMP also reduced the pretransition peak below resolvable limits. These changes are consistent with both acyl chain interaction and head group interaction within the lipid membrane. Intercalation of CTMP into the lipid membrane acyl chain region would give a signature much like that of the control compound ibuprofen, i.e., a lower T_m and reduction in enthalpy. Typically, acyl chain interactions are not associated with a change in the pretransition temperature or enthalpy, but it is possible that the temperature associated with the pretransition peak has been reduced as well, moving below the range tested (20 to 50 °C). The branched structure of CTMP would suggest that if the compound penetrated into the acyl chains, considerable disorder would be expected. It is possible that the acyl chain disordering is so extensive that the headgroup region is altered as a secondary effect, and not necessarily because of a direct surface interaction with CTMP. This hypothesis would explain why the thermal signature displays characteristics of both strong acyl chain interactions (depressed main transition and enthalpy), and molecular changes around the head group (loss of the pretransition peak). Repeated scans were consistent indicating that the lipid vesicles remained intact.

The salt CBEH, which has a branched alkyl chain anionic structure and a Log EC_{50} value <2.47 μM, exhibited much more complex

thermotropic behavior when DPPC vesicles were exposed to CBEH at a concentration of 10 mM. For each of the compounds investigated, including the controls, thermal reversibility was tracked by allowing the samples to cool back to the starting temperature following the initial heating scan and then reheating for at least 5 scans. With the exception of CBEH, for all of the compounds tested at a concentration of 10 mM, the gel to liquid-crystal phase transition was reversible with less than a 5% reduction in enthalpy upon reheating (data not shown). The initial heating scan of DPPC in the presence of 10 mM CBEH underwent a reduced pretransition near 33 °C, and a reduced main transition near 41 °C, with a reduction in enthalpy of 82 and 99%, respectively, compared to DPPC in HEPES alone (Table 1, Fig. 2B). A third peak was observed at 27 °C which was much higher in enthalpy than that observed for the peaks at either 33 or 41 °C making it difficult to observe all three peaks plotted to the same scale (Fig. 2B). That is, in the presence of 10 mM CBEH, DPPC underwent three phase transitions with four possible lamellar phases observed across the first temperature scan when starting at 20 °C and heating to 50 °C where the expected transitions at 33 and 41 °C were significantly reduced. Further, only the first heating scan yielded these results. The second through fourth reheating scans after cooling back to the initial temperature resulted in a reversible but altered dispersion where the phase transition first observed at 27 °C was reduced, and there was no resolvable main transition at 41 °C. It is possible that there was a uniform population of vesicles that underwent all of these transitions in the first scan, but more likely there was a mixed population of vesicles present with varying degrees of CBEH incorporation that ultimately converged to a uniform population after repeated thermal scanning.

To better understand the thermal signatures in the DPPC–CBEH samples, additional preparations were scanned over a wider temperature range from 10 to 90 °C (Fig. 2C). DPPC control samples (without added organic salts) scanned across the same temperature range changed little from those that were scanned from 20 to 50 °C, where the predominant feature is the strong main transition observed at 41 °C with a less pronounced pretransition at 36 °C (Fig. 2C). When scanning DPPC–CBEH (10 mM) samples over the larger temperature range a certain level of thermal annealing was apparent as the rescans (scan 2 and up) exhibited a change in peak distribution compared to the first heating scan where a new transition occurred at 20 °C and the 27 °C transition was reduced (data not shown). Others have reported a DPPC subgel crystalline bilayer (L_c) phase transition at 20 °C followed by transition into the gel phase at 27 °C depending on how the lipid was hydrated [24]. The DPPC batch solution was incubated for the same temperature and time period as all the other batch solutions, and there were no indications of the presence of annealing changes in the presence of any of the other compounds.

Additional DSC runs were included for preparations of DPPC in the presence of CBEH in concentrations lower than 10 mM in order to determine if the differences in thermotropic behavior were concentration dependent, as might be expected based on toxicity data. As can be seen in Fig. 2C, the extent of disruption of the membrane, as measured in this case by peak depression and broadening, was found to be directly proportional to concentration. After passing through an initial heating scan with a $T_m = 40.64 \pm 0.07$ °C, $\Delta T_{1/2} = 2.12 \pm 0.00$ °C, reducing the CBEH concentration to 0.1 mM resulted in a reversible main peak transition with a $T_m = 40.61 \pm 0.16$ °C and ($\Delta T_{1/2} = 1.68 \pm 0.15$ °C) ($n = 3$) (Fig. 2C). After passing through an initial heating scan with a $T_m = 36.81 \pm 0.33$ °C and $\Delta T_{1/2} = 1.59 \pm 0.00$ °C, reducing the CBEH concentration to 1.0 mM yielded a reversible main peak transition with a $T_m = 36.41 \pm 0.41$ °C and $\Delta T_{1/2} = 2.26 \pm 0.19$ °C ($n = 2$) (Fig. 2C). All concentrations of CBEH investigated significantly reduced the presence of the pretransition peak at approximately 36 °C normally associated with DPPC lipid vesicle preparations.

4. Conclusions

In the current study we investigated the interaction of a family of choline salts, which had been previously evaluated for toxic cellular interactions, with model membranes prepared from DPPC, a major phospholipid in macrophage membranes. Our studies used calorimetric methods to monitor changes in the gel to liquid-crystalline transition of DPPC, as this phase change is very sensitive to molecules that interact with the membrane. We were able to establish that organic salts with high toxicity profiles drastically altered the lipid phase change behavior of model membranes. These studies revealed that the phase transition behavior of a lipid membrane exposed to choline-based organic salt solutions can be dramatically affected by the structure of the anion. This has implications not only for designing biocompatible salts, but also for any biotechnology application in which membrane interaction is important.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bbamem.2013.03.017.

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