ChemComm

Cite this: *Chem. Commun.*, 2011, **47**, 12613–12615

www.rsc.org/chemcomm

COMMUNICATION

Zwitterionic sulfobetaine lipids that form vesicles with salt-dependent thermotropic properties†

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Received 19th September 2011, Accepted 13th October 2011
DOI: 10.1039/c1cc15804j

We describe a class of zwitterionic sulfobetaine (SB) lipids with fascinating salt-dependent properties. SB lipids are zwitter-neutral across a broad pH range; however they have negative surface potentials in the presence of anions and two salt-dependent transition temperatures. These new SB lipids provide insight on the role of charge orientation at the membrane interface and may be useful components in drug delivery systems.

Zwitterionic lipids such as phosphatidylcholine (PC) and phosphatidylethanolamine (PE), are the major lipids in the membranes that compartmentalize most organisms from microbes to man. Although there is a large literature on PC, PE, cationic and non-ionic lipids, there are surprisingly few studies on modifications to diacyllipids that retain a zwitterionic neutral headgroup at pH 7.4.[1–6] Therefore, there is a vast chemical space to explore for synthetic lipids that might shed light on the physical properties of membranes formed from non-biological lipids or provide new components for liposome-based therapies.

Here, we describe a novel class of zwitterionic SB lipids that differ structurally from PC lipids in two ways—the locations of the charged headgroup moieties relative to the bilayer, and the nature of the anionic group. SB lipids have an anionic sulfonate that extends into the aqueous media and a cationic amine adjacent to the bilayer, whereas for PC lipids, the anionic phosphate is adjacent to the bilayer and the cationic amine extends from the liposome surface (Fig. 1a). The SB lipids have interesting salt-dependent phase transition temperatures (T_m) and liposome-forming properties.

To our knowledge sulfobetaine diacyllipids have not been reported, although a variety of single chain sulfobetaine surfactants are well characterized and used in a variety of applications.[7–9] Investigations into the properties of SB surfactants in the presence of various salts reveal that anions bind preferentially over cations at the micelle interface. In the presence of salt, the otherwise neutrally charged micelles gain an overall negative charge.[10,11] Marte[11] reports that anions bind to the interface in the following order $OH^- < Cl^- < Br^- < ClO_4^-$, which parallels the Hofmeister series.[12]

Hydration of a dried film of the SB lipids in 150 mM NaCl at a temperature above the T_m of the lipid did not lead to the formation of liposomes. Based upon the findings in the surfactant field we surmised an inner-salt was forming either within the same headgroup or between neighboring headgroups and these interactions were interfering with liposome formation. We increased the NaCl concentration to disrupt the inner-salts and found that small diameter vesicles formed at NaCl concentrations greater than or equal to 500 mM (See ESI, Table S1). When the counter ion was changed from Cl^- to ClO_4^-, I^-, or Br^- at 150 mM, liposomes formed regardless of the acyl chain length (See ESI, Table S2). Zeta potential measurements of DMSB in the various salt solutions revealed the surface potential of the liposomes varied according to the counter-ion, $ClO_4^- > I^- > Br^- > Cl^-$, specifically: −38, −35, −23, −6 mV respectively, suggesting a higher degree of anion binding in the same order. These results show that as the polarizability of the anion in solution increases, so does its ability to interact with the liposome surface. Lipid films rehydrated in 500 mM NaF, did not form liposomes. We verified the formation of liposomes by transmission electron microscopy (TEM) (Fig. 1b), which reveals small, uniform diameter liposomes consistent with the dynamic light scattering data (see Fig. 1 legend).

Since SB liposome formation and stability is largely dependent on salt composition and concentration, we determined if the salt concentration/type influenced the T_m of the lipid colloidal dispersions (Fig. 2). We discovered two primary T_m for each SB lipid; a low T_m similar to that of its PC lipid acyl chain analog, and a high T_m similar to its PE lipid counterpart

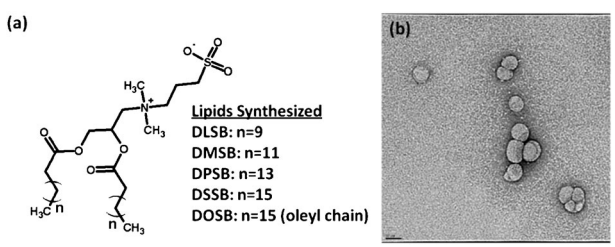

Fig. 1 (a) SB lipid structure. (b) TEM Images of DPSB/cholesterol: 10/3 mole ratio liposomes. Scale bar is 50 nm. Dynamic light scattering measurements indicated liposome diameters to be 70 nm.

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† Electronic supplementary information (ESI) available. See DOI: 10.1039/c1cc15804j

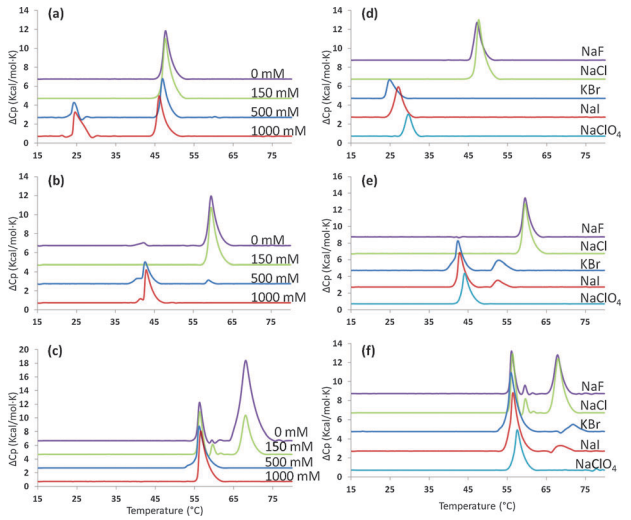

Fig. 2 Representative DSC traces for DMSB, DPSB, and DSSB (a–c) at four NaCl concentrations (a) DMSB, (b) DPSB, (c) DSSB and (d–f) with 150 mM NaF, NaCl, KBr, NaI, and $NaClO_4$ (d) DMSB (e) DPSB (f) DSSB. Repeated measurements have similar thermograms (See ESI, Table S5).

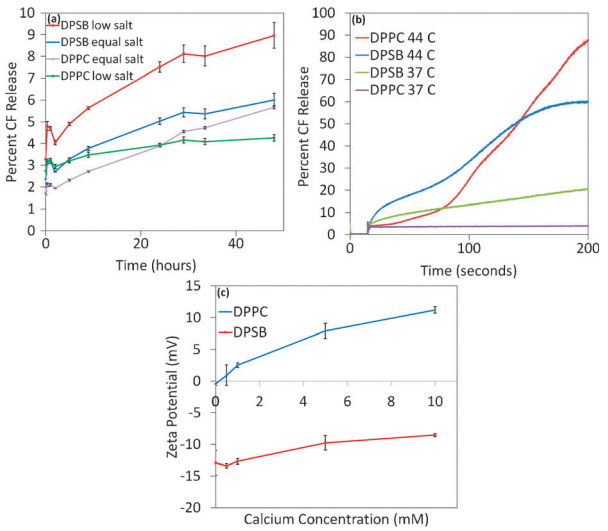

Fig. 3 (a) CF release from DPSB and DPPC liposomes both with 23% cholesterol. (b) Thermal induced CF release from DPSB and DPPC liposomes. (c) The effect of Ca^{2+} on the zeta potential of DPSB and DPPC liposomes with cholesterol as in (a).

(See ESI Table S4). The more polarizable anions (Br^-, I^-, and ClO_4^-) and high NaCl concentrations ($\geq$ 500 mM) favored the low T_m, while low NaCl concentrations and less polarizable anions (Cl^- and F^-) resulted in high T_m. The transition from high to low T_m appears to be a two-component process with intermediate transitions only observed in DSSB at 150 mM NaCl.

The orientation of the fixed charge at the bilayer interface is thought to influence the permeation of ions and charged molecules across the interface.[13] Thus, we compared the leakage rate of a model water soluble anion, carboxyfluorescein (CF), from SB or PC liposomes[14] in two buffers with salt concentrations either equal to or lower than the liposome preparation buffer. If the orientation influences ion permeation, one would expect CF to leak more rapidly from the SB liposomes. The CF release rates for the DPSB and DPPC formulations shown in Fig. 3a are slow and similar to each other in the "equal salt" buffer, suggesting only a small effect from the charge orientation. DPSB liposomes have a higher rate of release in the "low salt" buffer which may be due to the dissociation of Cl^- from the liposome surface, resulting in the formation of an inner-salt form and the subsequent destabilization of the bilayer.

The CF release rates for DPPC liposomes are similar for both buffers, which suggest a decreased sensitivity to changing salt conditions relative to DPSB liposomes. DPSB liposomes are fairly stable even when added to a buffer with a lower salt concentration and may be useful for encapsulating and delivering an aqueous drug.

In liposomal drug delivery, temperature induced leakage can allow for site-specific delivery of encapsulated material at a location where the temperature has been elevated above normal human body temperature (37 °C).[15,16] To examine the SB liposomes for potential use in thermally-triggered systems, CF release from DPSB/cholesterol/PEG-DSPE (85 : 10 : 5 mole ratio) liposomes was monitored at three different temperatures; physiological (37 °C), near the lower T_m of DPSB (44 °C) (Fig. 3b) and at the higher T_m of DPSB (59 °C) (See ESI, Fig. S3). DPSB liposomes have a more rapid release than DPPC liposomes at 37 °C and do not release to the same extent as DPPC liposomes at 44 °C. Since the T_m of the SB lipids are salt dependent we investigated if we could trap the liposomes in one T_m state by preparing them in either NaCl or KBr. We also took liposomes prepared in both salt types and exchanged the outside salt type with the other salt to determine the influence of a salt asymmetry across the bilayer on the thermal release properties (See ESI, Fig. S3). We did not observe substantial differences in CF release profiles from DPSB liposomes for any of the different salt conditions. The most significant differences in release across the various salt conditions were observed at 59 °C; however, none of the preparations showed steady release (similar to what was observed at 44 °C) at 59 °C, only an immediate initial release after which leakage stopped.

In biological systems, divalent cations are frequently present and can interact with lipid headgroups to induce aggregation, fusion, or alter the surface potential.[17] We measured the effect of Ca^{2+} concentration on the aggregation and zeta potential of SB liposomes. DMSB liposomes in the presence of up to 300 mM Ca^{2+} did not aggregate whereas DMPC liposomes aggregated at 300 mM Ca^{2+} (data not shown).

As previously reported,[18] the surface potential of the PC liposomes becomes increasingly positive as the Ca^{2+} concentration increases (Fig. 3c). The surface potential of the DPSB formulation also becomes more positive with increasing Ca^{2+} concentration, however the magnitude of the change is less and the zeta potential remains negative up to 10 mM Ca^{2+}. This indicates that Ca^{2+} has a weaker interaction with the SB sulfonate, which extends into the aqueous media, than with the PC phosphate anchored at the hydrophobic interface. The ability of SB liposomes to avoid aggregation and remain negative in the presence of elevated Ca^{2+} could be beneficial for drug delivery applications.

The salt-dependent properties of the SB lipids may be explained by the occurrence of two salt forms; an inner-salt formed within the same (or between neighboring headgroups)

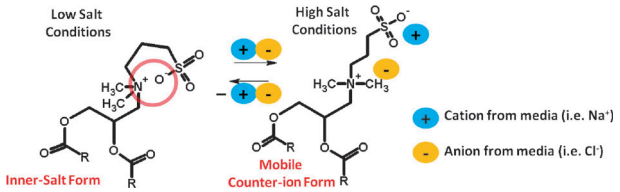

Fig. 4 Illustration of inner-salt and mobile counter-ion salt.

and a mobile counter-ion salt formed between the SB headgroup and ions in the surrounding media (Fig. 4). These two forms may be responsible for the two T_m observed for the SB lipids.

There are two possible explanations for the increased T_m—an increase in the strength of the interactions either between: (1) neighboring headgroups or (2) adjacent acyl chains. Increased acyl chain packing could occur for the case of the self inner-salt due to a decrease in headgroup surface area at the bilayer interface, allowing the hydrophobic chains to pack more closely. This would also lead to a higher T_m. However, replacing the quaternary amine in PC with either a methyl or t-butyl group, which decreases the headgroup area, does not increase the T_m.[19] This demonstrates that a decrease in headgroup size alone is not enough to produce an elevated T_m.

We think it more likely that the observed increase in T_m for the SB lipids is due to strong ionic headgroup interactions. Phospholipids, such as PE[20] and phosphatidic acid (PA),[21,22] have smaller headgroups and elevated transition temperatures relative to PC, but their increased T_m are attributed to hydrogen bonding interactions and not a decrease in headgroup surface area.[19,22] The hydrogen bonding hypothesis is supported by the decrease in the PE T_m to PC-like values at high pH, where the amine is deprotonated. PA lipids have a maximum T_m around pH 4, where the pK_1 hydrogen is 50% deprotonated and the hydrogen bonding ability across the liposome surface is maximized.[22] At pH 11, where the phosphate is almost completely deprotonated, the T_m of PA liposomes is more than 25 °C lower than at pH 4.

Interestingly, PC lipids with a three carbon spacer between the phosphate and quaternary amine,[19] similar to the SB lipid headgroup, do not show the same sort of behavior. Therefore, either the interaction of a quaternary amine with a sulfonate instead of a phosphate or the location of the quaternary amine adjacent to the bilayer promotes the inner-salt interactions.

In the mobile counter-ion salt form, the SB headgroup should become more hydrated and exhibit increased degrees of freedom. This would increase the surface area at the bilayer interface and disrupt the attractive headgroup interactions.

In conclusion, a new class of zwitterionic SB lipids has been synthesized that exhibit salt-dependent properties that differ from PC lipids. The SB lipids have an inverse charge orientation at the membrane interface and may provide new ways to create membranes with adjustable, asymmetric properties. SB lipids could also serve as a useful tool for studying membrane biophysics and properties important to drug delivery, such as surface potential and encapsulated ion release.

This work was supported by the National Science Foundation Graduate Fellowship Research Program and By NIH grants EB003008; GM061851 and a UCSF-QB3 Pfizer grant.

Thanks to Reena Zalpuri at UC Berkeley's Electron Microscope Lab for her assistance with the TEM experiments and to Colin Walsh, Vincent Venditto, and Aditya Kohli for helpful discussions.

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