Release Rates of Liposomal Contents are Controlled by Kosmotropes and Chaotropes

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Abstract

Contents release from redox-responsive liposomes is anion specific. Liposomal contents release is initiated by contact of apposed liposome bilayers having in their outer leaflet 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE), whose presence is due to redox-stimulated removal of a quinone propionic acid protecting group (Q) from Q-DOPE lipids. Contents release occurs upon the phase transition of DOPE from its lamellar liquid crystalline (L_α) to hexagonal-II inverted micelle (H_{II}) phase. Contents release is slower in the presence of weakly hydrated chaotropic anions versus highly hydrated kosmotropic anions and is attributed to ion accumulation near the zwitterionic DOPE head groups, in turn altering head group hydration, as indicated by the $L_\alpha \rightarrow H_{II}$ phase transition temperature, T_H, for DOPE. The results are significant, not only for mechanistic aspects of liposome contents release in DOPE-based systems, but also for drug delivery applications wherein exist at drug targeting sites variations in type and concentration of ions and neutral species.

INTRODUCTION

Lyotropic (Hofmeister)[1,2] cations and anions, as well as osmolytes, are present in time- and location-dependent concentrations within, and in the vicinity of, cancerous tumors and cells. For example, lactate concentrations in tumors are 2–4 times and bicarbonate values 4–6 mM higher that in plasma.[3] In addition, intracellular/extracellular Cl^-, CO_3^{2-}, and glucose concentrations vary with tumor type,[4] and elevated Na^+ concentrations are found in brain and breast tumors.[5]

Stimuli-responsive drug delivery vesicles based on phospholipids have been identified for the treatment of a variety of diseases, and have received great attention for improving cancer therapy, due to the ability of 70–200-nm diameter liposomes to accumulate at diseased sites, thereby decreasing the side effects and improving the efficacy of the drug contained within.[6] During in vitro assessment of the capabilities of various responsive liposome systems, the solution conditions used for their evaluation—particularly salt and buffer identity and concentration—vary widely between individual studies. Comparison of contents release characteristics for liposome systems in different laboratories is challenging, especially those systems whose success hinges on interliposomal or membrane–liposome, lipid–lipid events.

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Phosphoethanolamine (PE) lipids are highly targeted for use in stimuli-responsive liposomes, due to their ability to promote liposome contents release at a selected site, typically via liposome bilayer-bilayer (aggregation/contents dispensing) or liposome bilayer-cell membrane (hemifusion/fusion) interactions that are predicated on the PE liquid crystalline lamellar to inverted hexagonal phase transition ($L_\alpha \rightarrow H_{II}$).[6] However, to date, the possible influence of *any* salt or osmolyte (Hofmeister species) on the *kinetics* of PE-based liposome contents delivery has not been addressed. Thus, it is of great value to gain fundamental knowledge about the influence of salt identity and concentration on the contents release process that is based on the PE $L_\alpha \rightarrow H_{II}$ phase transition.

We have chosen to evaluate the impact of salt nature on liposome contents release using the Q-DOPE lipid-based liposome system we have developed, Scheme 1, because initiation of release is anticipated to occur at a number of cancer tumors that over express NAD(P)H:quinine oxidoreductase isozyme 1 (NQO1), known to specifically reduce quinones.[7] In addition, contents release from reduced liposomes composed of Q-DOPE occurs by contact-induced lysis of liposomes possessing in their outer leaflet a critical amount of the H_{II} phase-prone DOPE, which is generated upon cleavage of the reduced quinone moiety, Scheme 2.[8, 9] Upon formation of the critical number of PE lipids to allow for liposome aggregation[10] and bilayer apposition at experimental temperatures T_{exp} T_H, the $L_\alpha \rightarrow H_{II}$ transition of the PE occurs,[11] leading to burst-phase contents release.[8, 9]

Based on observations by Petrache[12] and Leontidis[13] with multilamellar phosphatidylcholine (PC) bilayer assemblies and those by Seddon[14] and Quinn[15] for PE bilayer assemblies, we anticipated the presence of anions having varying polarizabilities/hydration levels would influence the aggregation and contents release from reduced Q-DOPE liposomes in a manner dictated by the Hofmeister series.[1] This hypothesis is founded on conclusions, drawn from experimental[12, 13] and theoretical[12, 16] outcomes for the PC layers above, that point to the accumulation of ions near the head group region of the lipids, as a result of ion partitioning events or non-electrostatic dispersion forces[17] acting between the ions and the lipids. Theoretical studies indicate the existence of anion concentrations as high as seven times that of the bulk value,[18] which can in turn result in anion adsorption at the lipid head groups for more polarizable anions,[13, 19] a change in interfacial water order/density,[18, 20–22] and dehydration of lipid head groups.[12, 13, 16]

RESULTS AND DISCUSSION

Displayed in Figure 1 are the time-dependent contents release, aggregation (light scattering), and zeta (ζ) potential measurements for ~120-nm Q-DOPE liposomes reduced by dithionite.[23] The ion specificity of the contents release process is borne out when comparing the three aforementioned observables for reduced Q-DOPE liposomes in the presence of 0.075 M KSCN (Figure 1A) and 0.075 M KCl (Figure 1B).[24] The time required for initial aggregation/contents release[25] t_{init} and that for 50% maximum contents release[8] $t_{50\%}$ are significantly larger for the Q-DOPE liposomes in the presence of the more polarizable/less hydrated SCN^- (44 min, 98 min) in comparison to those for the less polarizable/more hydrated Cl^- (34 min, 42 min).[26] The amount of DOPE in the outer leaflet for Q-DOPE in the presence of KCl at $t_{init} = 34$ min is 53% of the maximum possible, while it is 62% for the KSCN at $t_{init} = 44$ min,[23] pointing to the requirement of more DOPE exposed to achieve aggregation and subsequent contents release in the presence of the more polarizable thiocyanate. For both anions, the zeta potential ζ is approximately −20 mV at the point of initial aggregation/contents release, having become more positive than the statistically equivalent initial ζ of −50 ± 1 mV (SCN^-) and −51 ± 2 mV (Cl^-) at $t = 0$. The similarity in ζ values for the two anions at t_{init} points to the following possibilities: no appreciable anion binding or poor experimental precision/low frequency of ζ measurement during the lengthy

in situ experiment. We suggest the latter need to be improved; the difference in ζ for 100% PC liposomes in 0.5 M KBr and KNO_3, or KI and KBr is roughly 3 mV,[12] while it is 4 mV for 80% PC:20% phosphatidic acid liposomes in 0.15 M NaBr versus NaCl.[27]

To explore further the ion specificity of the contents release process, a select group of Hofmeister anions with a common cation (K^+) were used, Figure 2. The t_{init} values increase with increasing polarizability (chaotropicity) of the anion in solution, while there is a decrease in the initial release rate (slope of the release curves,[28] which directly correlates with the rate of aggregation, as noted in Figure 1) during the burst-phase process, Table 1. Times for initial liposome aggregation/contents release follow the inverse Hofmeister series ($SCN^- > NO_3- > Br^- > Cl^- > SO_4^{2-}$), with the initial release rate tracking the direct Hofmeister series, under salt concentrations where electrostatic interactions between approaching H_{II}-prone lipid layers are minimal (double layer ~0.4 nm). Furthermore, Q-DOPE liposomes that have not been reduced are stable indefinitely in the presence of 0.500 M salts. Thus, the observed ion-specific events are due to characteristic properties of outer leaflet DOPE under the influence of aqueous anions.

The H_{II} phase of PE lipids is energetically favored[29] with low amounts of interfacial water at the phosphoethanolamine moiety, because of the decreased lateral pressure in the head group region (smaller head group/increased hydrogen bonding/reduced spontaneous radius of curvature); while at higher water values, the larger PE head group prefers the expanded L_α phase.[15] Thus, the T_H of the $L_\alpha \rightarrow H_{II}$ transition of PEs increases with increased head group hydration, and decreases with decreased hydration; the sensitivity of this effect can be as large as 7 °C per water of hydration.[14]

We posited that well-hydrated, less polarizable anions (kosmotropes) will accumulate near the DOPE head group region and dehydrate the DOPE so as to cause a decrease in the T_H of DOPE, while accumulation of weakly hydrated, more polarizable anions (chaotropes) near the head group will tend to increase T_H. This hypothesis is based on experimental work with PC bilayers wherein head group hydration level is anion specific.[13, 19] Surprisingly, no calorimetric data exist for DOPE in the presence of different Hofmeister series salts. The measured T_H of DOPE follows the inverse Hofmeister series ($SCN^- > NO_3^- > Br^- > Cl^- > SO_4^{2-}$), with the more polarizable/less hydrated anions increasing T_H, and those that are less polarizable/more hydrated lowering T_H, in comparison to the case of no added KX salt, Table 2. These data parallel that for 1-palmitoyl-2-oleoyl-phosphoethanolamine (POPE) in the presence of kosmotropic and chaotropic agents and that of PEs at different levels of head group hydration.[14, 15]

Based on the work of Aroti and Leontidis concerning ion accumulation near PC head groups that leads to their ion-specific dehydration,[13, 16] a similar event for DOPE in the reduced Q-DOPE liposomes would result in increased intermolecular hydrogen bonding between neighboring phosphate and protonated ethanolamine groups when in the presence of less polarizable anions. Larger intermolecular hydrogen bonding exists in the much less hydrated H_{II} phase of PEs versus the disordered L_α phase.[14, 30] A salt-induced, dehydration-driven increase in DOPE inter-lipid association would be in accord with the larger inter-lipid interactions found for POPE bilayers in the presence of NaCl,[31] and the anion-specific lipid head group hydration of DOPC bilayers that follows the inverse Hofmeister series.[19] Furthermore, it has been shown that the ability of apposed lipid bilayers to approach each other and make initial bilayer-bilayer contact (stalk formation) at a critical separation distance is dictated by *only* the work required to dehydrate the apposed lipid head groups.[32] Thus, it is expected that hydration of DOPE in the reduced Q-DOPE liposomes will be decreased in the presence of salt, and the hydration level will follow the inverse Hofmeister series. Such a scenario will result in increased time to initial liposome aggregation (t_{init}) for

increasing DOPE hydration caused by accumulation of more polarizable/less hydrated anions, while the initial rate of contents release will correlate with the direct Hofmeister series, because the H_{II} phase will be favored in the presence of less polarizable kosmotropes that dehydrate DOPE. This model is in agreement with our observations.

The results from the work at hand may also be explained, in part, by the impact of salt identity on the interbilayer water thickness between apposed bilayers, a characteristic designated as a measure of interbilayer repulsion in multilamellar PC stacks that follows the inverse Hofmeister series.[12, 13] Our t_{init} data agree with the inverse Hofmeister *trend* for this model, and also the unusual behavior for the thiocyanate case, which could be attributed to direct interaction of this special chaotropic anion with the DOPE head group region. Anion binding that gives rise to head group expansion that favors the L_α phase of DOPE should result in an increased t_{init} due to electrostatic repulsion between approaching liposomes, and also a decreased rate of contents release. However, the rate of release for 0.5 M KSCN (4.8 ±0.1 % min^{-1}) is *larger* than in the absence of added KX salt (3.5 ±0.1 % min^{-1}). In addition, we find that t_{init} is *decreased* when any salt is present ($t_{init}=$ 59 min for no added salt). Our observations are counter to the increase in repulsion found upon salt addition for the PC systems.[12, 13]

OUTLOOK

The outcomes from the work here point to its importance and that of future work aimed at gaining additional fundamental knowledge about the influence of salt anion/cation and osmolyte identity and their concentration on contents release from this and other PE- or diacylglycerol-based liposome systems targeted for drug delivery applications. As stalk formation is the key step in the $L_\alpha \rightarrow H_{II}$ transition[32] for contents release from stimuli-responsive Q-DOPE liposomes[8, 9] and others,[6] it may be possible to gain additional knowledge about ion-specific effects using strategies that lead to stabilization of aggregated liposomes, stalk structures, and hemifused liposomes.[9, 32] In addition, it has been shown that the $L_\alpha \rightarrow H_{II}$ transition of di-dodecylphosphatidylethanolamine is exceedingly sensitive to the presence of short-chain carboxylate anions, even becoming *inaccessible* at sufficiently high carboxylate concentrations.[14] Thus, the elevated lactate/lactic acid concentrations in the interstitial fluid of cancer tumors[3, 4] may have a profound influence on the way in which therapeutic liposomes release their drug contents. In addition, clathrin-mediated endocytosis of drug delivery vehicles[33] has the potential be influenced by the presence of Hofmeister salts, noted by their impact on clathrin-vesicle stability.[34] Furthermore, sugars have a significant effect on the T_H of lipids,[35] which can also lead to the stabilization of cell membranes. Thus, the dynamic glucose concentration levels associated with tumors is anticipated to impact the pharmacokinetics of liposomal drug delivery. Finally, it will be valuable to learn if the presence of various ions and osmolytes leads to a change in the mechanism of liposomal contents release (burst release vs. leakage)[9] when the experimental temperature is below that of T_H of the lipid bilayer, as for SCN^- in Figure 2.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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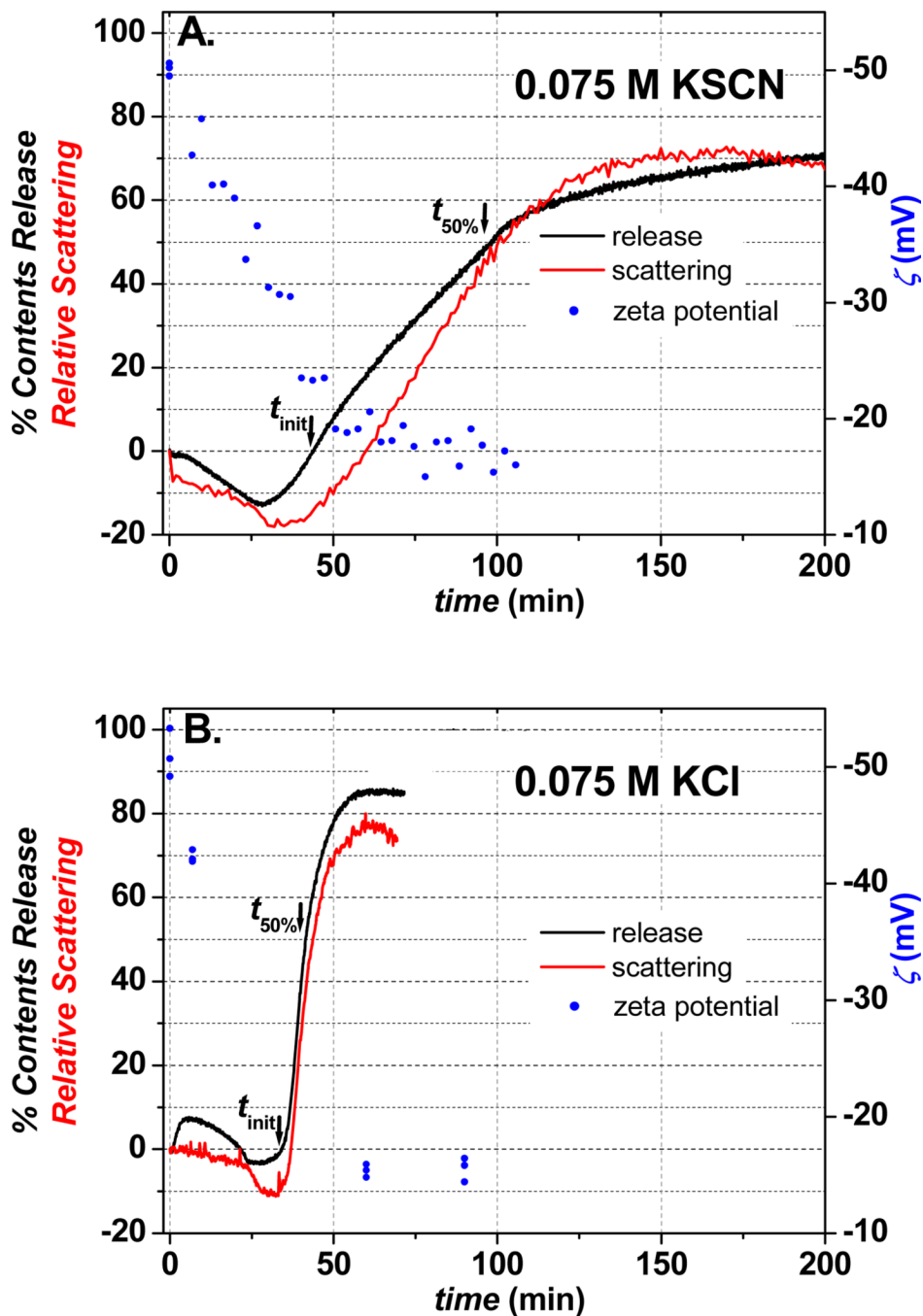
Figure 1.
Impact of anion identity (0.075 M) on liposome contents release process: (A) KSCN and (B) KCl. Release of self-quenched calcein, the relative light scattering response at 90°, and the zeta potential for ~120-nm diameter, 0.0975 mg mL^{-1} (100 μM) 100% Q-DOPE liposomes after their reduction by 5 equivalents of the membrane impermeable dithionite, $S_2O_4^{2-}$, in pH 7.40, 0.050 M phosphate (potassium salt) buffer. $T = 25$ °C in all cases.
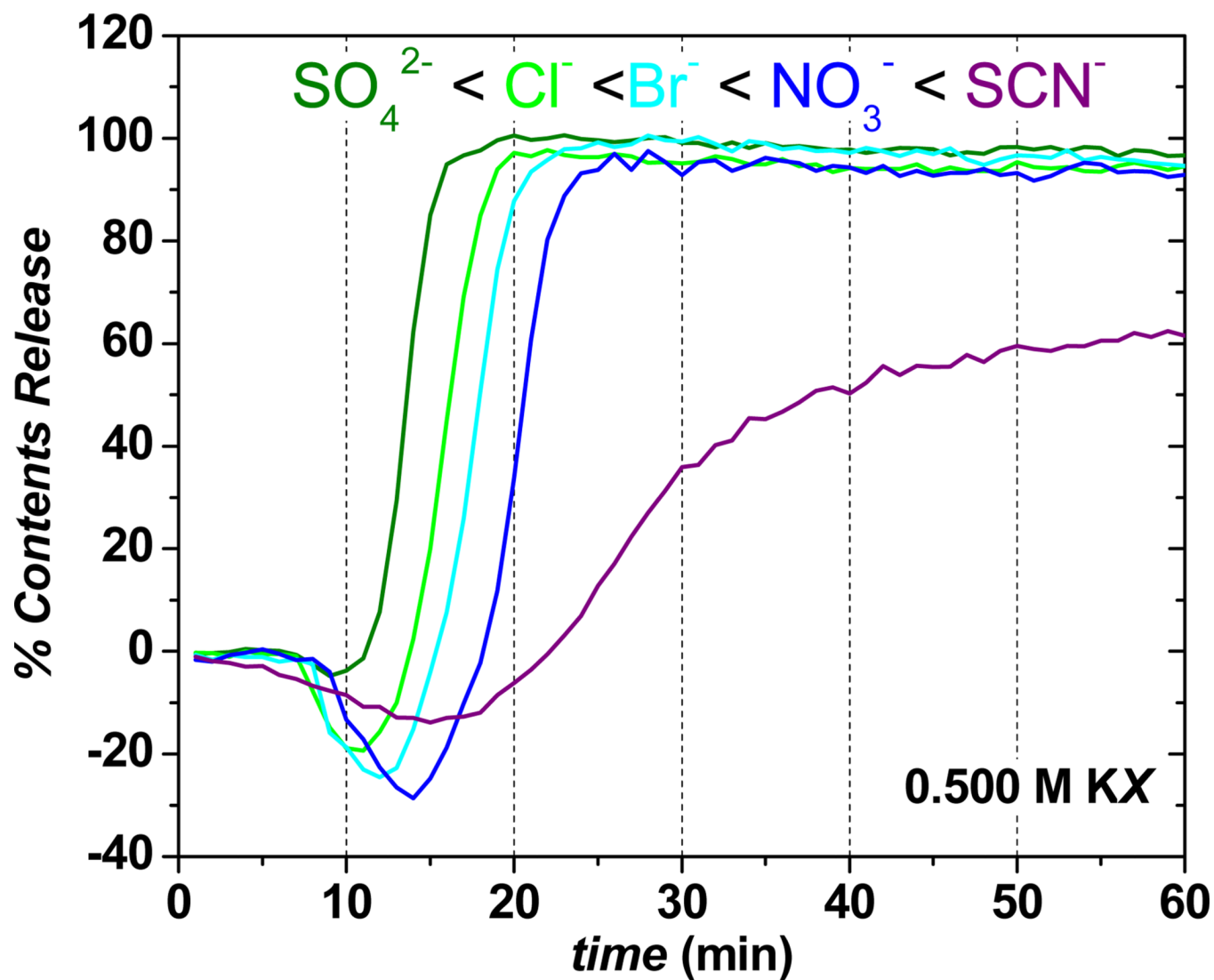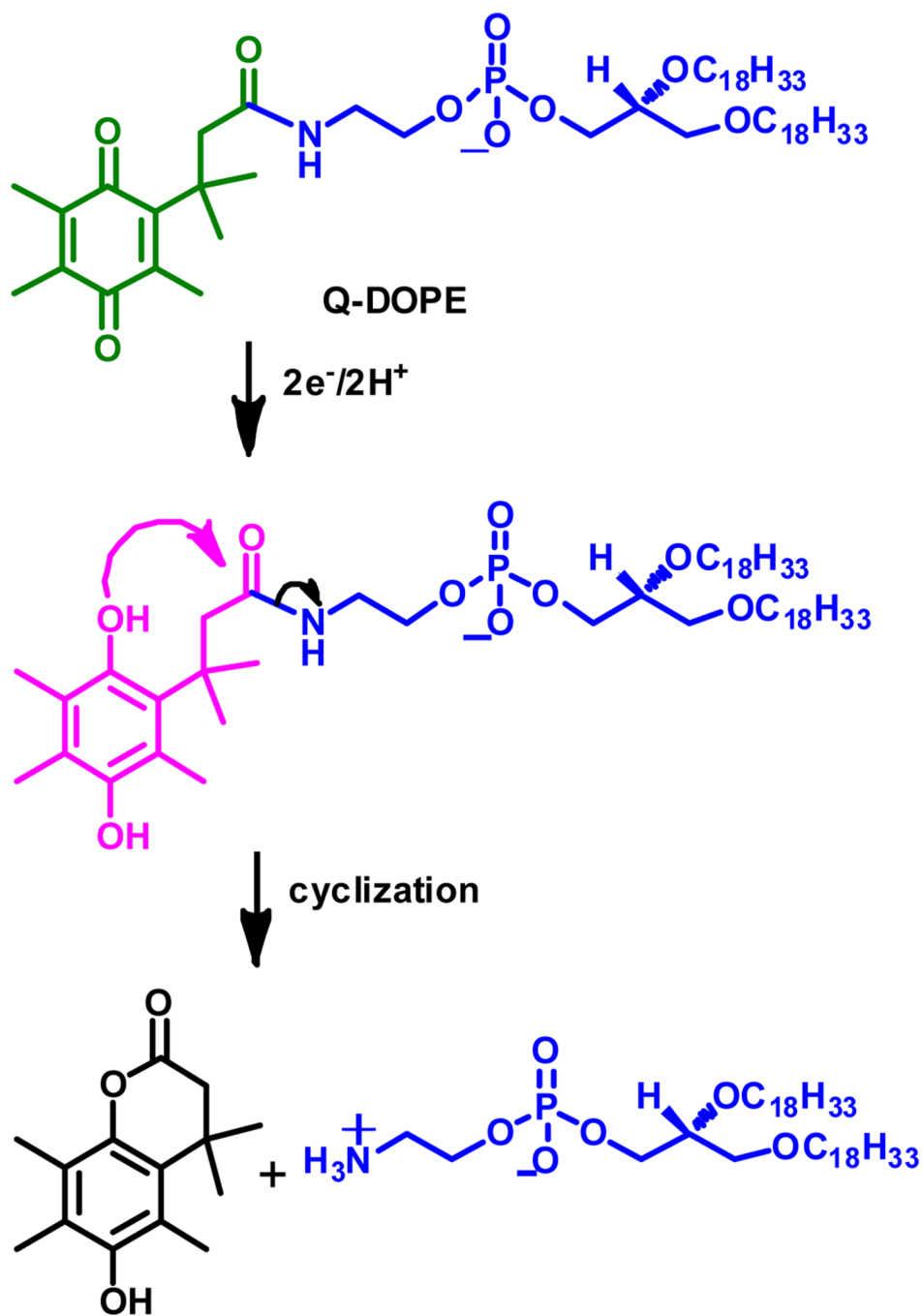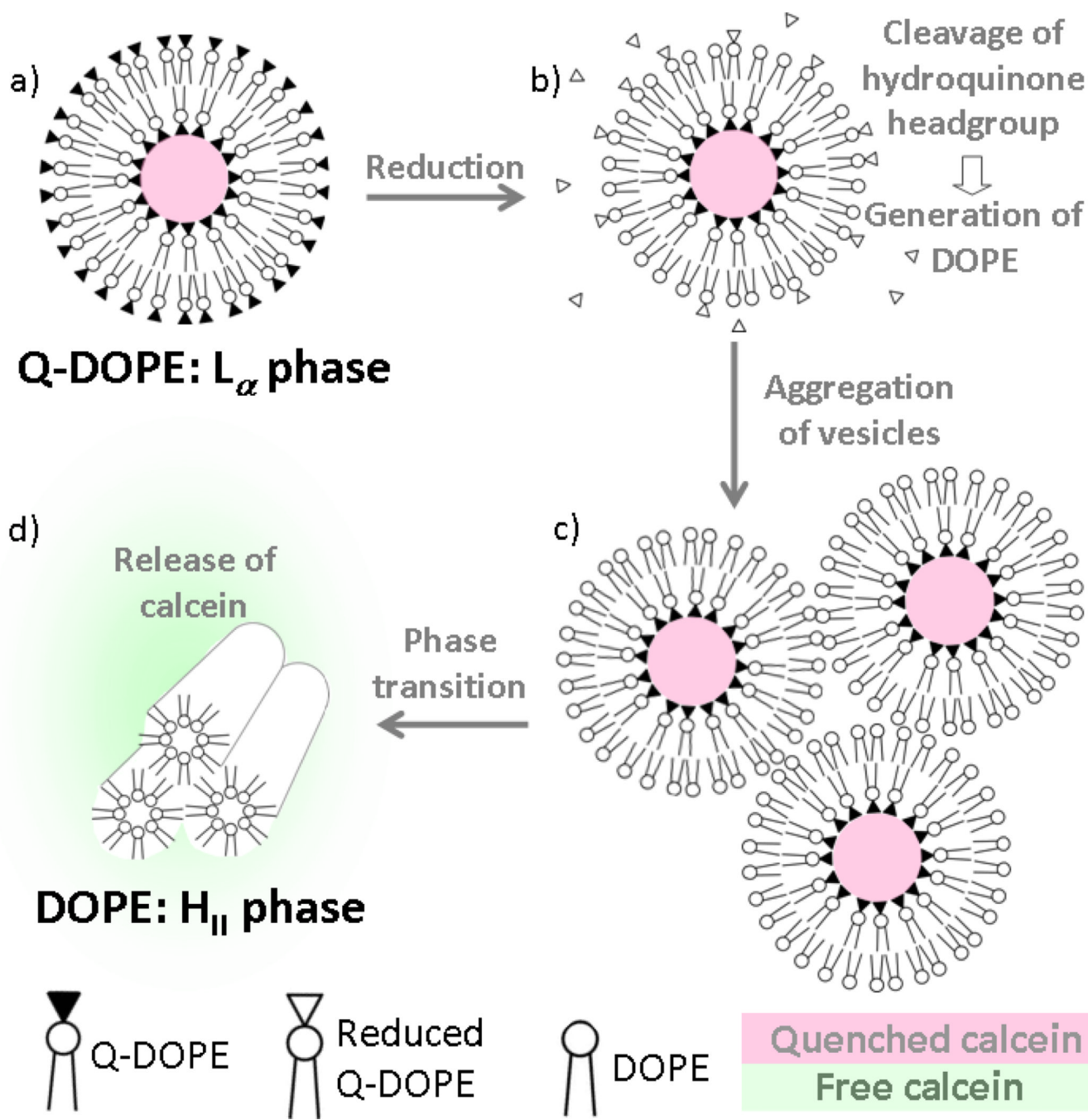
Figure 2.
Effect of anion (0.500 M) identity on contents release from 100% Q-DOPE liposomes in pH
7.40, 0.050 M phosphate (potassium salt) buffer after their reduction via addition of one
molar equivalent of dithionite, $S_2O_4^{2-}$. $T = 25$ °C in all cases.

Scheme 1.
Q-DOPE lipid, and its reduction and subsequent products

Scheme 2.
Q-DOPE liposome contents release

Table 1

Effect of Anion on Time Required for Initial Aggregation/Contents Release t_{init} and Initial Release Rate for Reduced Q-DOPE Liposomes in Figure 2 at 25 °C

Salt (0.500 M)	t_{init} (min)[a]	initial release rate (% min^{-1})[b]
K_2SO_4	12	26.5 ± 1.5
KCl	14	21.2 ± 2.1
KBr	16	15.0 ± 1.9
KNO_3	19	11.0 ± 1.8
KSCN	2	4.8 ± 0.1

[a]Uncertainties are ± 1 min.

[b]Average with ± 1 standard deviation.

Table 2

Calorimetrically determined T_H values for DOPE (1.88×10^{-2} M) in pH 7.40, 0.050 M phosphate buffer with added 0.500 M salt

Salt	T_H (°C)[a]
K_2SO_4	8.8 ± 0.4
KCl	9.8 ± 0.5
KBr	13.3 ± 0.4
None	13.1 ± 0.5
KNO_3	15.6 ± 0.6
KSCN	36.7 ± 0.8

[a]Average with 1 standard deviation for three replicates.