COMMENTS

Comment on "Gel-Fluid Transition in Dilute versus Concentrated DMPG Aqueous Dispersions"

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The thermal behavior of the negatively charged phospholipid dimyristoyl phosphatidyl glycerol (DMPG) has gained increasing interest over the past decade.[1−11] Under conditions at which the surface charge density is high, this lipid shows an intermediate phase between the gel and fluid phases, with particular features, such as low turbidity and high viscosity.[5] In the intermediate phase, the average bilayer microviscosity steadily decreases as the temperature is raised, monitored by fluorescent and spin labels.[8,9] The heat capacity shows a sequence of endothermic peaks between a very sharp one at T_m^{on} (around 18 °C), corresponding to the beginning of the chain melting process, and a broader one at T_m^{off} (around 30 °C), coinciding with its end.[8] At increasing salt concentrations or decreasing pH values, as the phosphate groups are screened and/or neutralized, the intermediate phase vanishes, and the main phase transition ultimately occurs at a unique temperature T_m, which depends on the ionic strength and phosphate−ion affinity.

In the paper commented here,[9] we discussed changes in the thermal behavior of DMPG caused by lipid dilution. We observed that below a certain DMPG concentration c' (∼0.4 mM, at low ionic strength) the intermediate phase vanished, being replaced by a much narrower transition, centered at a single T_m value, which increased from 27 °C to 42 °C as the lipid concentration was decreased from 0.1 to 0.01 mM DMPG (Figure 1a). Based on experiments with NaCl and pH variations, we suggested that this effect could be caused by an increase in proton affinity for lipid concentrations below c', though, as discussed in the paper, the reason for this increase was rather unclear. However, in contrast to this hypothesis, recent experiments discussed here show that this increase in T_m is actually caused by contamination from small amounts of divalent cations, possibly calcium, present in the solutions.

Figure 1b shows differential scanning calorimetry (DSC) runs obtained with 0.1 mM DMPG with and without 0.05 mM EDTA. The sample without EDTA shows a peak centered at 27 °C, similar to that in Figure 1a. The addition of the calcium-chelating agent EDTA restores the DMPG thermal behavior to what we called high concentration regime (above $c' \sim 0.4$ mM), with the complex calorimetric DSC profile, the fingerprint of the intermediate phase. This implies that the high T_m value observed with dilute DMPG dispersions is caused by the binding of divalent cations, possibly calcium, present in the solution, which is relevant only when the lipid is diluted. It is important to say that EDTA (up to 3 mM) did not affect the thermal behavior of 1 mM DMPG dispersion (above c'). Higher EDTA concentrations significantly lowered the sample pH, thus inducing an increase in T_m (data not shown).

To further prove the hypothesis that the increase in T_m upon lipid dilution was driven by calcium binding, DSC runs of DMPG above c' (namely, 1 and 10 mM DMPG) were performed at different PG/Ca^{2+} molar ratios. Figure 1c shows the runs obtained with 1 mM DMPG at different amounts of added $CaCl_2$. The DSC runs obtained with 10 mM DMPG at the same PG/Ca^{2+} molar ratios were quite similar (results not shown). Upon the addition of 0.05 mM $CaCl_2$ (PG/Ca^{2+} molar ratio of 20:1), the intermediate phase vanishes and a peak centered at 27 °C is observed. Further addition of $CaCl_2$ increases T_m to 42 °C at 0.5 mM $CaCl_2$ (2:1 molar ratio), in accordance with values previously reported in the literature.[12−15] The similarity between the DSC series in Figures 1a and 1c is striking, further supporting the hypothesis that calcium binds to the DMPG bilayer surface below c'. Figure 1d shows that the addition of 0.5 mM EDTA to 1 mM DMPG + 0.05 mM $CaCl_2$ also restores the thermal behavior to what was obtained before calcium addition. On the basis of the results shown, we estimate around 0.005 mM Ca^{2+} contamination in the solution.

In the commented paper,[9] we also showed that the thermal behavior of DMPG did not change upon lipid dilution when working at high ionic strength. The reason for this is now clear. By increasing the NaCl concentration, the small amount of divalent ions present becomes irrelevant.

Although the commented paper[9] reported mainly the effects of DMPG dilution, which now turns out to be an effect of changing the PG/Ca^{2+} molar ratio (or other divalent cations), the paper added important achievements to the characterization of DMPG, mainly the phase diagrams of temperature versus lipid concentration (Figure 4 in ref 9) and versus NaCl concentration (Figure 6a in ref 9), for DMPG concentrations above c'. Having in mind that the vanishing of the intermediate phase below $c' \sim 0.4$ mM DMPG was due to the binding of calcium ions present in our solution, it is now clear that the intermediate phase extends to lipid concentrations well below c', if the experiments are performed in the presence of EDTA.

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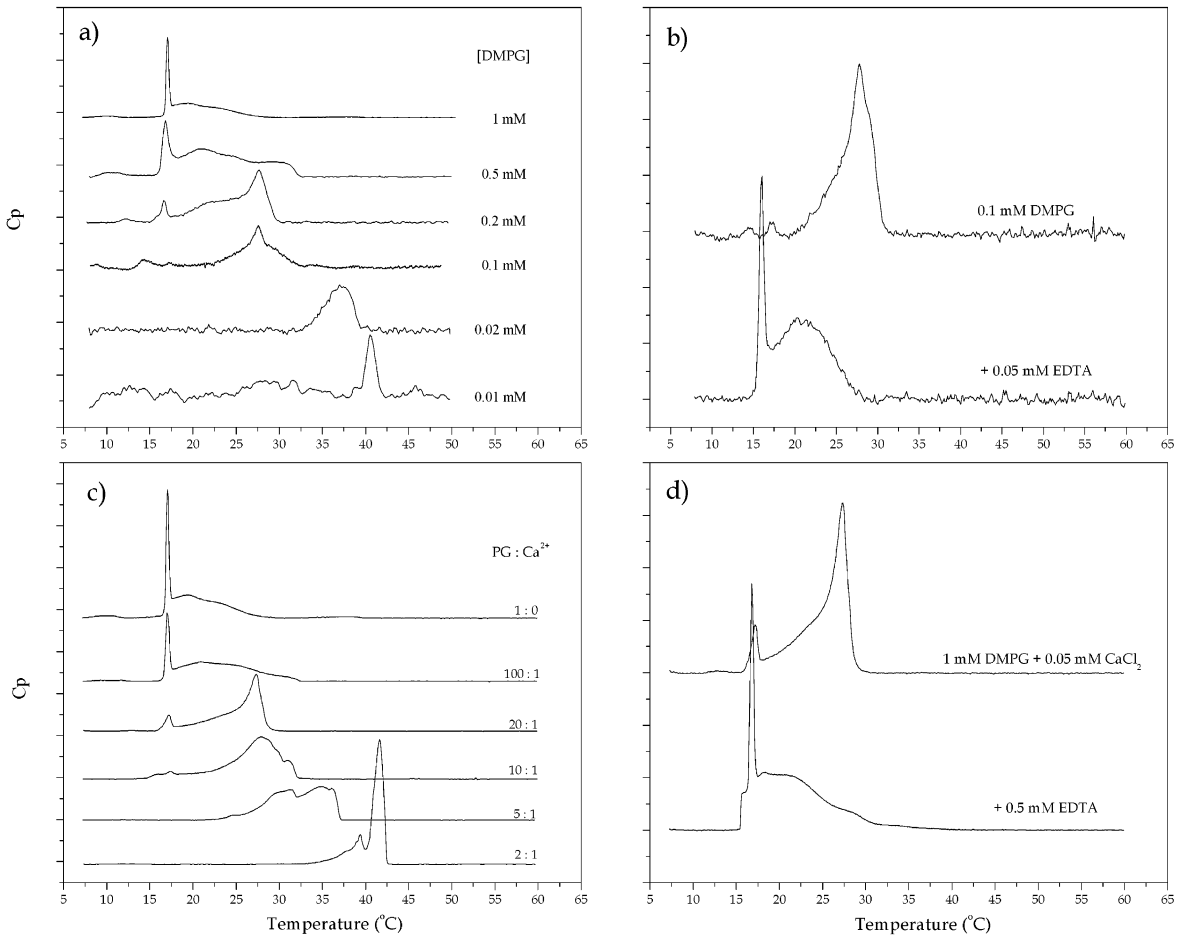

Figure 1. (a) DSC runs of different DMPG concentrations in 10 mM HEPES pH 7.4 + 2 mM NaCl (same as Figure 2 in the commented paper[9]). (b) DSC runs of 0.1 mM DMPG in 10 mM HEPES pH 7.4 + 2 mM NaCl with (bottom) and without (top) the addition of 0.05 mM EDTA. (c) DSC runs of 1 mM DMPG in 10 mM HEPES pH 7.4 + 2 mM NaCl in the presence of different amounts of $CaCl_2$. The PG/Ca^{2+} molar ratios are indicated in the figure. (d) DSC runs of 1 mM DMPG in 10 mM HEPES pH 7.4 + 2 mM NaCl + 0.05 mM $CaCl_2$ with (bottom) and without (top) the addition of 0.5 mM EDTA. Scan rates: (a) and (b) 60 °C/h; (c) and (d) 30 °C/h.

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