DHPC-DMPC-water phase diagramme at high water content

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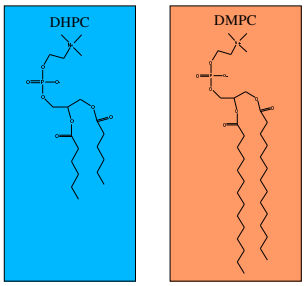
Introduction

The mixture of the short-chain, micelle forming, phospholipid dihexanoyl phosphatidyl choline (DHPC) and the membrane forming phospholipid dimyrisoyl phosphatidyl choline (DMPC) is of particular interest since the mixture is able to form a magnetically alignable « bicelle » phase of use for solid state nmr studies of membrane associated proteins and crystallisation of some membrane proteins.
However the physical organisation of these mixtures is the matter of some debate. In this poster we present our data on the phase diagramme and the structures of the different phases.
We have restricted ou measurements to 27°C which is slightly above the DMPC gel-liquid phase transition temperature.

The protagonists



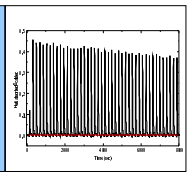
Solubility of DHPC in membranes

To investigate the solubility of DHPC in DMPC membranes we have measured by Isothermal titration calorimetry (ITC) the heat signature of mixing liposomes with a dilute solution of DHPC. The concentration of this solution was insufficient to cause membrane perforation. The thermogram below gives us an estimate of the detergents water-membrane partition enthalpy and free energy.

$K_{water->membrane} = 47.8\ M^{-1}$

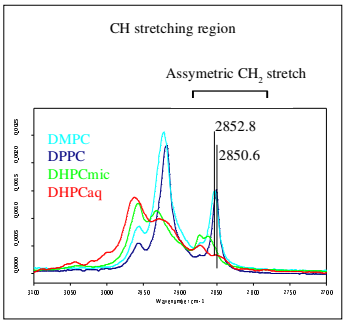
$\Delta G° = -9.6\ kJ\ mol^{-1}$
$\Delta H° = 12.4\ kJ\ mol^{-1}$
$\Delta S° = 73\ J\ mol^{-1}\ K^{-1}$

compare with cmc of 9.5mM, K.cmc = 0.454



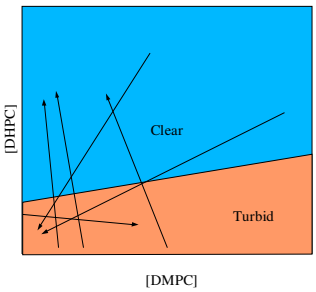
Acyl chain order

Infrared spectroscopy can be used to measure the organisation of the acyl chains in DHPC/DMPC mixtures. We therefore measured spectra of several different mixtures of DHPC and DMPC. These spectra indicate that DHPC insertion into DMPC bilayers causes an increase in lipid chain order – in marked contrast to wht is observed for most detergents. The acyl chains become disordered only at very high DHPC/DMPC ratios.



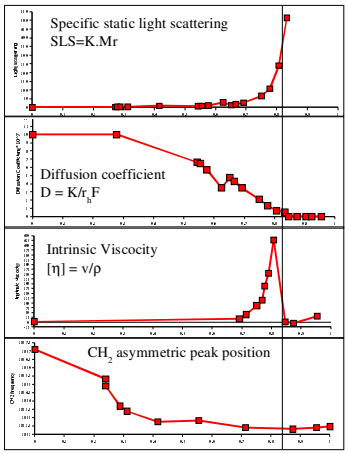
Procedure

To study the phase diagramme and obtain information on the structure of the different phases we have made a number of different type of measurement on different mixtures of DMPC and DHPC. In the diagramme above we show the various titrations we have performed positioned with respect to the main turbid/clear transition. During these titrations we have measured different physical parameters – heat release (ITC), turbidity, static light scattering (SLS), dynamic light scattering (DLS), viscosity, and the infrared spectrum.



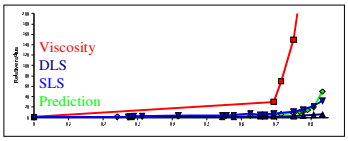
Solution phase structure

The different measurements we have made allow us to examine the physical properties of different compositions of the aggregated phase.



Observed and calculated evolution of radius, assuming a bicelle model

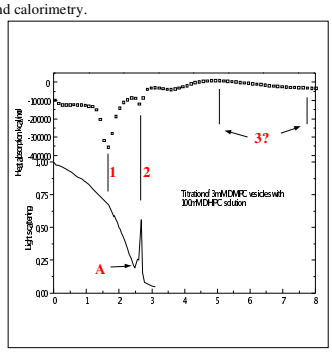
The two light scattering and viscocity measurements each allow an estimation of bicelle radius in a system where falling DHPC/DMPC ratio yields larger bicelle disks of constant thickness – furthermore this radius can be predicted from the composition of the phase.



While the theory and SLS measurements coincide approximately the diffusion coefficient falls much to slowly while the viscosity rises much too fast. However the changes in viscocity are not expected for a perforated bilayer phase.

Membrane solubilisation

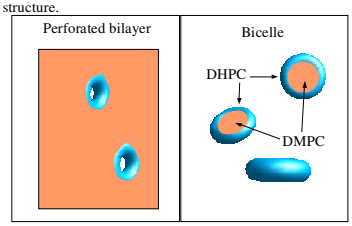
We have followed the disolution of 100nm DMPC liposomes with DHPC by turbidimetry (light-scattering) and calorimetry.



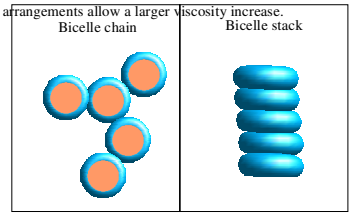
The first event (1) coincides with the release of encapsulated material, we interpret this as membrane perforation. The second event (2) we attribute to solubilisation of (perforated) membranes. The weak third thermal event (3) might correspond to the formation of mixed micelles. The point (A) shows where a hazy viscous phase, possibly corresponding to perforated bilayers, is observed.

Conclusions

During dilution of lipid mixtures we see no evidence for a transition to a perforated bilayer phase as has been postulated from various previous experiments. However we do appear to see this phase transiently during membrane solubilisation, point A, where a hazy viscous phase precedes a slow transition to the clear viscous phase. It is unclear from our experiments if this perforated bilayer structure represents a stable phase existing over a very limited range of compositions or a kinetically trapped structure.



The stable clear viscous solution we observe regularly close to the clear/turbid phase transition, during different types of titration appears not to arise from clasical bicelles or perforated bilayers. A possibility is the formation of stacked bicelle disks or chains of bicelles, these arrangements allow a larger viscosity increase.



To confirm this hypothesis further experiments are necessary, in particular to measure carefully the concentration dependance of viscocity and light scattering at fixed condensed phase composition.

We hope to be able to use our knowledge of this phase diagramme to study lipid-protein and protein-protein interactions in bicelles of known dimensions

We also hope to extend these studies to investigate bicelle-bicelle interactions in order to beter understand how concentrated bicelle solutions can orient in magnetic fields.

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