Density measurements through the Main (L_α - $P_{\beta'}$) and "Pre" ($P_{\beta'}$ - $L_{\beta'}$) Phase transitions of di-hexadecanoyl - phosphatidylcholine - John Jones, Gordon Tiddy, Leo Lue and Alberto Saiani (J.Jones@postgrad.manchester.ac.uk, Gordon.Tiddy@manchester.ac.uk, Leo.Lue@manchester.ac.uk, A.Saiani@manchester.ac.uk)

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Introduction

Lipids are increasingly gaining importance as a basic component of innovative biotechnological and pharmacological applications. It is of little surprise then that lipid bilayers have been much studied as the prototypical membrane. A main focus of such studies is the structure of lipid bilayers and how the lipid bilayer (different makeup of lipids in the bilayer) properties are affected by its environment [1, 2].

The use of density measurement to observe the volume change over the main and pre-transitions is a technique seldom used. However, when coupled with other non evasive techniques, such as calorimetry (microDSC), it becomes a powerful tool that provides some fascinating insights into the phase behaviour of lecithin bilayers. Usually, these observations are used to estimate either the volume of the lipid molecules or the energy associated with a phase transition.

Taking this approach to the next level, we can use the two techniques to calculate volume occupied by each CH_2 group and specific internal energy changes over phase transitions. The energy changes over the main transition can be largely attributed to van der Waals and rotameric energy changes, the sum of which is approximately equal to the enthalpy change of the main transition. Both the volume and energy change per CH_2 group can be estimated by comparison to the volumes and vaporization energies of normal alkanes - utilising the invaluable DETHERM database.

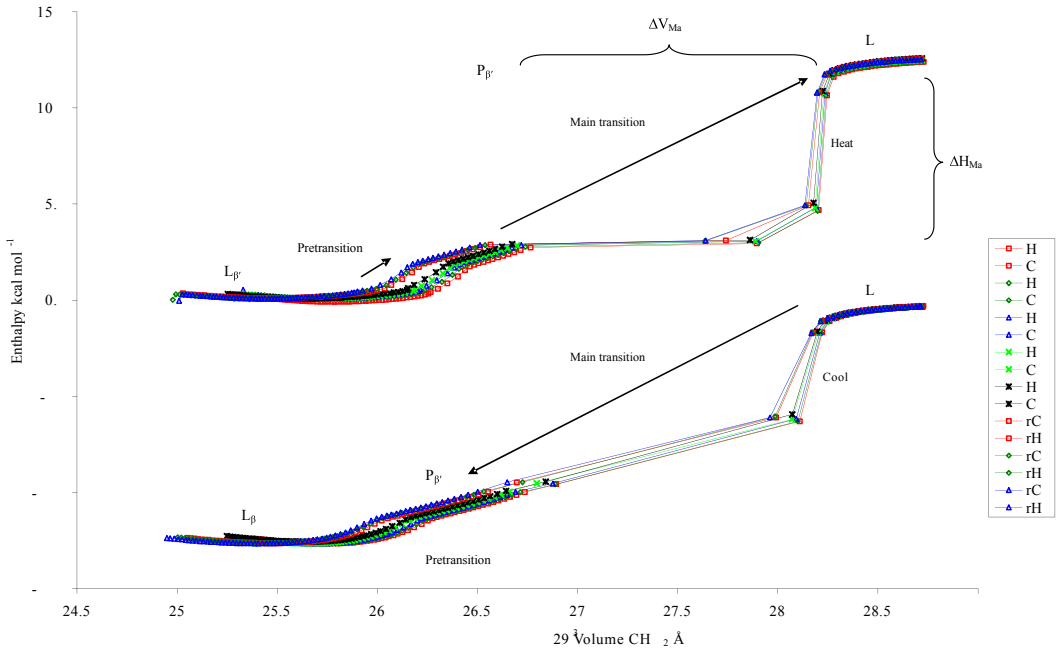

Figure 1 A chart showing enthalpy as a function of CH_2 group volume. The curves show measurements for a sequence of five heat/cool cycles (H1, C1, H2, C2, H3, C3, H4, C4, H5, C5) after initial equilibration at 5°C for 24h. The sample was then held at 50°C for 24h followed by three heat/cool cycles (reC1, reH1, reC2, reH2, reC3, reH3. The volume (ΔV_{Main}) and enthalpy (ΔH_{Main}) change over the main transition are highlighted.

Experiments & Results

Experiments on several lecithins, chiefly di-hexadecanoyl-phosphatidylcholine, have been carried out using the density meter (Anton Paar DMA 5000) and microDSC (Setaram microDSC III) [3] and have revealed a large unreported volume hysteresis phenomenon. By manipulating the volume and microDSC data in thisway, it is possible to directly compare the volume and energy changes as a function of temperature and gain some remarkable insights into the mechanism behind the phase transitions.

Some hysteresis in volume per CH_2 group between heating and cooling is clearly visible in Figure 1, existing predominantly in the low temperature gel phase. This phenomenon is believed to be an effect of the continual development of low volume gel phases through successive temperature cycling. Eventually the hysteresis does reach an equilibrium value at which further heat/cool cycling no longer changes the volume. Remarkably, we also observe pretransitional behaviour in the L_α phase before the main transition, suggesting the presence of gel-like lipid patches, as has been found in other research on biological membranes. These appear to result in the formation of a different gel phase on subsequent cooling cycles.

In this analysis, the volume per CH_2 group was found to be in remarkably good agreement with work proposed in Refs. [1, 4] and in computational models [5]. But interestingly, the van der Waals energy change over the main phase transition was found to be smaller than the rotameric energy change; quite the reverse of the results in Ref. [1]. Here again, the DETHERM database has provided invaluable reference data with which detailed theoretical calculations can be performed. Importantly, we can also estimate the van der Waals energies of other molecules provided the volume change per CH_2 group is known.

In the future we plan to investigate the influence of other components (e.g. cholesterol, fatty acids) on these phenomena. We anticipate that having ready access to the DETHERM database as is provided by the EPSRC Chemical Database Service will play a major role in the selection of specific materials.

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