Hydrated/Dehydrated Lipid Phase Transitions Measured Using Nanocalorimetry

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ABSTRACT: The phase transition evolution with hydration of a model system, 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), was investigated with a fast nanocalorimetry system. Using nanocalorimetry, it is possible to measure the gel to liquid phase transitions that occur on millisecond to second time scales and quantify the time to recover the hydrated state. The results show the phase transition occurring in a few milliseconds and the relaxation or recovery time from the dehydrated state back to original hydrated state occurring with times dependent on the local humidity. With relative humidity (RH) of 43% or higher, the recovery time can be less than a few seconds. With RH of 11% or lower, the recovery time is extended to greater than a minute. The recovery process is controlled by mechanisms that depend on the lipid molecular repacking and water transport from the environment. Nanocalorimetry provides a powerful method to investigate the kinetics of such transformations in lipids and other biological and pharmaceutical moieties. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:3442–3447, 2014

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INTRODUCTION

Lipids and lipid membranes are studied to understand their basic properties and behavior and to harness their properties in applications ranging from drug delivery[1] to sensing.[2,3] Of course, in natural cells, lipids are closely associated with water and the role of hydration in lipid structures is beginning to be understood.[4] Lyophilizing, or freeze drying, is well-known method to preserve a hydrated molecular structure after water removal; such preserved moieties can later be reconstituted for use, including drug delivery, protein-based drugs, and vaccines.[5–7] To investigate the role of water in molecular structure and stabilization, the interactions between lipids and water have been measured by conventional differential scanning calorimetry (DSC), small-angle X-ray scattering (SAXS), fourier transform infrared spectroscopy (FTIR), and neutron diffraction.[8–11] Some biological transformations of interest occur within only a few nanoseconds to seconds; time scales too short for investigations with conventional thermal analysis tools, but within the capabilities of nanocalorimetry. Our recent review outlines other applications of thermal analysis for a variety of biological materials.[12]

Ambient relative humidity (RH) effects on diffusion in supported lipid monolayers have been reported.[13] In physiological water-rich conditions, the polar head groups are hydrated and the water molecules disrupt the van der Waals interaction between each head group.[14]

Microfabricated nanocalorimeters are capable of thermal measurements on samples with very small mass (micrograms to nanograms) and at fast rates.[15–17] Nanocalorimetry is widely used and has been applied for measurements with a variety of types and forms of materials.[12,18] A typical nanocalorimeter sensor can heat at rates up to 10^5 K/s and has sensitivity, in terms of heat capacity, on the order of 1 nJ/K. Because the sample can be heated and cooled so quickly, nanocalorimeters allow measurements of multiple cycles at short intervals providing opportunities to probe lipid hydration, dehydration, and rehydration. Previous work using a scanning liquid calorimeter (operating at 1 K/s) has shown different degrees of phase transitions of dimethyl dioctadecyl ammonium bromide, but such heating rate is slower than the transition times being studied.[19,20]

In this paper, we report on humidity-dependent phase transitions of a model system, 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC). Gel to liquid phase transitions were measured under varied levels of RH. With fast heating and cooling rates, the process of drying the DPPC film is observed over more than one thermal cycle, and the kinetics of water adsorption/desorption are studied.

EXPERIMENTAL

Chemicals

1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC, CAS 63-89-8) was purchased from Avanti Polar Lipids (Alabaster, Alabama). Trehalose was purchased from Sigma–Aldrich (St. Louis, MO).

Sample Preparation

1,2-Dipalmitoyl-sn-glycero-3-phosphocholine was dissolved in ethanol at a concentration of 5 mg/mL. A fully hydrated DPPC sample was stabilized by mixing trehalose/DPPC (molar ratio, 1:1) in water at a concentration of 10 mg/mL. The mixed solution was extruded to form liposomes by a mini-extruder (Avanti Polar Lipids) with 0.1 mm polycarbonate membrane filters (Whatman, Inc., Newton, Massachusetts). The solution was

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deposited over the nanocalorimeter sensor by electrospray.[21] The potential was 5 kV (the analog display of the power supply provides resolution of approximately ± 0.2 kV), the target distance was 2 cm, the solution flow rate was 0.2 mL/h using a syringe pump with a blunt 25 gauge syringe needle as an emitter tip.

Nanocalorimetry Measurements

The fabrication and calibration of the nanocalorimeter sensors used in this work has been described in detail previously.[22] For this work, each sensor was calibrated in an oven over the range of 20°C – 80°C and the temperature-resistance relationship recorded. After sample deposition, the sensors were transferred to the measurement apparatus with well-controlled RH and allowed to reach equilibrium over a period of 48 h. Measurements were performed by applying a current pulse and measuring the current and the voltage drop to provide an instantaneous measure of chip resistance and power. Based on the temperature coefficient of resistance from calibration, chip temperature is calculated at each time point (5 μs intervals). The acquired data are post-processed to calculate apparent heat capacity by subtracting the power needed to heat the bare chip. The apparent heat capacity would include the heat capacity of the sample and the enthalpy of any transitions. Each thermal cycle was programmed with a heating period of 50 ms and a cooling period of 50 ms, the time between cycles was adjusted depending on the experiment.

RESULTS

Phase Transition Temperature of DPPC

1,2-Dipalmitoyl-sn-glycero-3-phosphocholine was deposited by electrospray, as shown in Figure 1a. The sample is uniformly deposited as droplets over the active area of the chip. For measurements with an interval (defined as the time from the end of one cycle to the beginning of the next cycle) of 200 ms (shown in Fig. 1b), the peak of the transition temperature increases with cycle numbers as the sample dries. As an example, for the data shown in Figure 1b, the first peak is 61.8°C and the last peak (10th cycle) is at 74.2°C. The transition temperature increased by 12.4°C over these measurements. It is worth noting that the incremental increase gets smaller with each cycle. Based on the reported enthalpy of bulk DPPC, 27.7 kJ/mol,[23] the mass of DPPC is estimated to be around 560 ng. With an estimated specific volume of 1 mL/mg, the film thickness is estimated to be 170 nm. The sample can absorb and desorb water very quickly and the sample hydration is an important aspect of the transition temperature. In order to clarify these measurements, subsequent work was undertaken on samples designed to represent the fully dry and fully wet state.

Phase Transition of DPPC—Achieving a Dry State

The dry state of DPPC was realized two ways—by purging dry nitrogen or by drying in a cryogenic chamber under high vacuum. As shown in Figure 2, after purging with dry nitrogen for at least 1 h, the transition temperature increased to 92.2°C. With thermal measurements at 300 ms intervals, only the first cycle shows lower temperature peaks (64.3°C and 90.5°C), which we believe is associated with tightly held water released during the first heating cycle of the experiment which illustrates the difficulty of obtaining a sample in the true dry state.

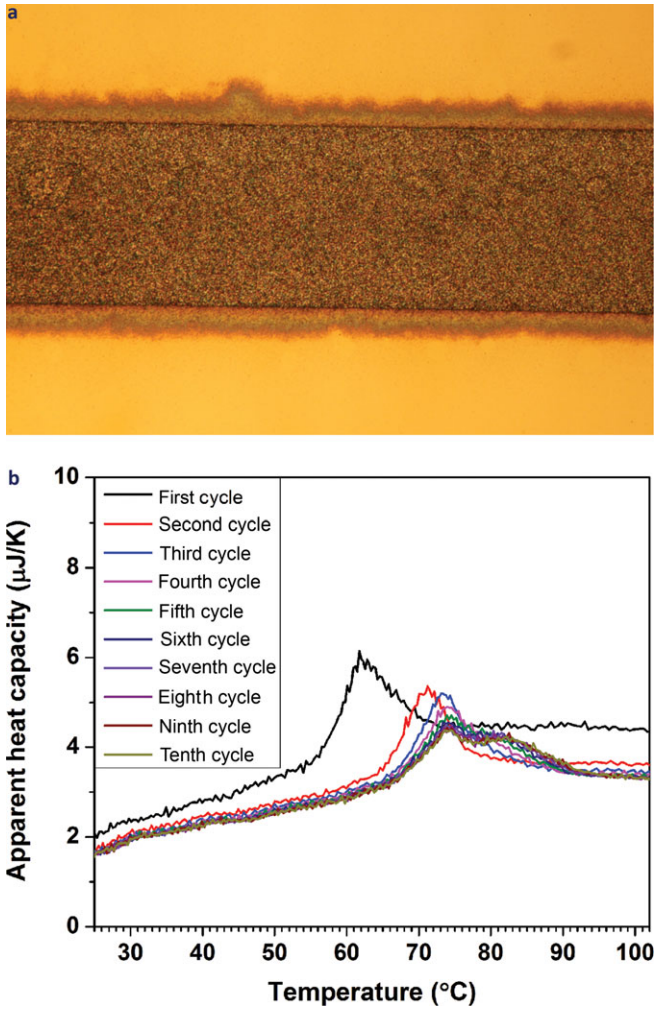

Figure 1. DPPC phase transition measured over 10 heating cycles in air atmosphere, the interval between cycles is 200 ms. (a) Sensor with deposited sample, (b) measured transition temperatures over 10 heating cycles.

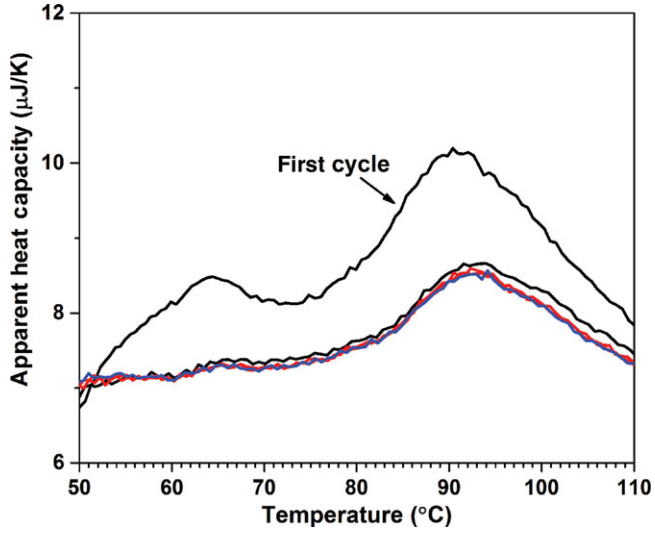

Figure 2. DPPC phase transition in dry state measured by nanocalorimeter in dry nitrogen, the interval time between cycles is 300 ms.