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Structural insights into the cubic–hexagonal phase transition kinetics of monoolein modulated by sucrose solutions

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Using DSC (differential scanning calorimetry), we measure the kinetics of the cubic–H II phase transition of monoolein in bulk sucrose solutions. We find that the transition temperature is dramatically lowered, with each 1 mol kg \(^{-1}\) of sucrose concentration dropping the transition by 20 °C. The kinetics of this transition also slow greatly with increasing sucrose concentration. For low sucrose concentrations, the kinetics are asymmetric, with the cooling (H II –cubic) transition taking twice as long as the heating (cubic–H II) transition. This asymmetry in transition times is reduced for higher sucrose concentrations. The cooling transition exhibits Avrami exponents in the range of 2 to 2.5 and the heating transition shows Avrami exponents ranging from 1 to 3. A classical Avrami interpretation would be that these processes occur via a one or two dimensional pathway with variable nucleation rates. A non-classical perspective would suggest that these exponents reflect the time dependence of pore formation (cooling) and destruction (heating). New density measurements of monoolein show that the currently accepted value is about 5% too low; this has substantial implications for electron density modeling. Structural calculations indicate that the head group area and lipid length in the cubic–H II transition shrink by about 12% and 4% respectively; this reduction is practically the same as that seen in a lipid with a very different molecular structure (rac-di-12:0 β-GlcDAG) that makes the same transition. Thermodynamic considerations suggest there is a hydration shell about one water molecule thick in front of the lipid head groups in both the cubic and H II phases.

1 Introduction

Monoolein is a deceptively simple lipid that exhibits a rich array of temperature and composition sensitive behavior. Lamellar, inverted hexagonal, sponge phases and minimal surface based cubic phases are all part of monoolein’s extensive and intriguing structural repertoire. 1,2 When one considers that it is inexpensive, biodegradable and so non-toxic as to be commonly used a food additive, the range of possible applications becomes immense. Monoolein and similar lipids are used as drug delivery systems, as matrices for membrane protein crystallization and even as templates for the creation of 3-D metal nanowire networks. The fascinating phase behavior and numerous applications have led to the thousands of papers and patents that study or utilize monoolein; consequently, this has led some to describe monoolein as a ‘magic lipid’. 3,4

In excess water, monoolein forms a minimal surface based phase (see Fig. 1) over a broad temperature range running from below room temperature to 90 °C. The minimal surface in this case is known as the D surface; it has cubic unit cell and can be

Fig. 1 Depictions of lipid phases. Lipid heads are shown as green spheres and the tails as black lines. Bluish-purple surfaces mark the lipid–water interface in the D surface based cubic phase; blue cylinders in the H II phase indicate the water cores.

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stacked three dimensionally. A lipid bilayer straddles the minimal surface and divides space into two identical but separate water channel networks. There are several minimal surface based phases with cubic unit cells. For simplicity’s sake, we will use cubic to refer to the D surface based phase in this paper. In depth dimensional and phase data can be found in Briggs et al.1 and Qiu and Caffrey;2 further information on minimal surfaces in general and electron density modeling and reconstruction of monoolein in particular are found in Harper and Gruner3 and Harper et al.4 Above 90 °C, monoolein transitions to the inverted hexagonal (HII) phase, which consists of water cylinders wrapped in lipid monolayers which are in turn stacked in a hexagonal pattern (see Fig. 1).

In depth structural studies have been performed on phospholipids that form the HII phase7–9 and monoolein itself in this phase has recently been the focus of molecular dynamics simulation.10

The non-lamellar structures seen in monoolein are seen not only in a variety of lipid systems, including lipids with PE (phosphatidylethanolamine) headgroups7,11 and PC (phosphatidylcholine) headgroups,12 but also in block copolymers.13,14 Non-lamellar phase behavior offers a deep window into fundamental processes such as membrane fusion.15–17 Furthermore, non-lamellar phase behavior has been useful in understanding the action of anti-microbial peptides.18–21 Several other examples of biological relevance can be found in Cook et al.22 Given the ubiquity, beauty and biological relevance of these phases, there has been considerable theoretical interest23–27 and this remains an active area of inquiry.

The cubic phase in monoolein is especially attractive for encapsulating drugs as it is stable both at room and physiological temperatures. It is quite viscous, allowing for the deposition of stable thin films. Since the cubic phase is a lipid–water matrix, both hydrophobic and hydrophilic drugs can be incorporated.28,29 A wide variety of drug release systems have been developed, including a tunable, pH dependent matrix30 and a magnetic field controlled system in which ferromagnetic nanoparticles are incorporated into the matrix.31

Another important application of monoolein is in the field of membrane protein crystallization.22,33 The cubic phases enable the formation of crystals in which the proteins are embedded in a lipid bilayer, a configuration much closer to the native state. It has long been known that membrane properties and composition deeply affect membrane protein function.34 Consequently, lipid–protein crystals offer a fruitful arena for examining the effects of membrane composition on proteins.35–37 There are also promising efforts to use these crystals to probe femtosecond time scale behavior of proteins.38

Given the rich array of structures formed by monoolein and the wealth of applications, there are a number of studies considering the effects of various additives.39–41 There also have been extensive investigations of cosmotropic and chaotropic solutes, especially sugars, on the fluid lamellar Lα to HII transition (see Koyanova et al.42 and references therein). Sugar–lipid interactions are particularly noteworthy as sugars are often used by living systems to stabilize their membranes; the detailed mechanism by which this occurs is an ongoing area of investigation.43 Surprisingly, there has been a relative dearth of work on sugar–monoolein mixtures, with most investigations focused on structure studies of isothermal mixtures.44–47 Our work starts to fill that gap by mapping out the phase diagram of monoolein with an excess of sucrose–water solutions at a variety of concentrations.

Phase transition kinetic studies and their analysis are challenging, but potentially quite rewarding. Phase transitions often involve the rupture and repair of membranes; hence, understanding the kinetics could ultimately reveal information about pore formation energetics and time scales. Often performed at X-ray synchrotrons, there have been several studies on cubic kinetics48–50 and some theoretical effort as well.51 We probe the kinetics of the cubic–HII phase transition in monoolein–sucrose–water mixtures using DSC (differential scanning calorimetry). Avrami theory, a powerful tool for phase transition analysis,52 has been brought to bear on lipid phases transitions involving lamellar phases53 and for the Lα–HII transition.22 In this work, we apply Avrami theory to a cubic–HII transition for the first time.

2 Materials and methods

Monoolein was obtained from Nu Check Prep (Elysian, MN) in powder form with a purity > 99% and sucrose was procured from Sigma Aldrich (St. Louis, MO) in polycrystalline form with a purity ≥ 99.5%.

2.1 Density measurements

The concentration of monoolein samples varied from 0.5–5 wt%, with the most successful samples prepared at < 1 wt%. Monoolein powder (~ 10 mg) and milliQ water (~ 1.2 g) were weighed on an analytical balance (Mettler AE 163). The sample was heated in an oven at 60 °C for 10 minutes and sonicated briefly, 3 times 3 s bursts, at room temperature. The sample was replaced at 60 °C for 10 minutes, resorced in short bursts, replaced at 60 °C for 30 minutes and resorced in short bursts. Care was taken not to cause foam on the top of the sample from the sonication. This sample preparation differed from the usual protocol of temperature cycling between 60 °C and ~20 °C with vortexing,54 since vortexing and cold temperature caused the lipid to clump. Samples with a monoolein concentration > 1 wt% also clumped more easily. The sample was then loaded into the DMA 5000M densimeter (Anton Paar) and held at 50 °C for three days prior to the first cooling scan. Six heating and cooling scans between 7 °C and 50 °C were performed with the density recorded at 0.5 °C increments. The heating scans occurred at a rate of 12 °C h⁻¹ and the cooling scans at 4 °C h⁻¹. Data were averaged using the Origin 8 software and the standard deviation was calculated.

2.2 DSC measurements

DSC (differential scanning calorimetry) measurements were made using a TA Instruments (New Castle, DE) Q20 differential
scanning calorimeter. Stock solutions of sucrose were prepared, with concentrations ranging from 0.5 to 3.0 molality in 0.5 molality increments and sonicated for at least 20 minutes to ensure mixing. Samples were made by using a 10 μL Drummond dispenser (Broomall, PA) to transfer about 1 mg of monoolein and about 10 mg of sucrose solution to a Tzero hermetic pan (TA Instruments). The pan was then covered with a Tzero hermetic lid and sealed using a Tzero press. A sealed sample pan in which a small hole was made in the lid was used as a reference pan, as is the recommended practice with this instrument. DSC runs consisted of multiple heating and cooling scans at rates of 0.2, 0.1, 0.05, 0.02, and 0.01 °C s⁻¹ over a range of temperatures that varied with the sucrose concentration and were in the region of about 20 °C to 90 °C. The sealed samples were weighed before and after the DSC measurements to check for leakage. If there was a deviation of more than a few hundredths of a mg between the weights, the sample was considered compromised and the data was not used. We found that under the repeated cycling, the likelihood of leakage strongly increased as temperatures neared 100 °C. The temperature of the cubic–H₂ phase transition for monoolein and pure water is 90 °C;² at the elevated temperatures necessary to measure the kinetics of this transition, we inevitably encountered sample leakage and so were unable to measure the kinetics for monoolein and pure water.

3 Results and discussion

3.1 Density measurements – experimental results
The volume of monoolein as a function of temperature is shown in Fig. 2.

The sample in that figure was scanned repeatedly, heating and cooling, for a total of six scans. In addition, four partial scans were carried out, confirming the full scans. Between each heating and cooling scan the sample was removed and replaced in the densimeter, since we previously determined that lipid settling over time can cause an artifactual increase in density in the DMA densimeter. The change of slope at 24 °C was quite reproducible. According to multiple phase diagrams of monoolein in water, there is no lipid phase transition near 24 °C at high hydration, such as in our sample. According to these phase diagrams, monoolein is in the Pn̅3m cubic phase plus excess water both below and above 24 °C. Therefore, the origin of this transition is not known.

3.2 Density measurements – comparison with literature
As seen in Fig. 2, the two values for the density of monoolein are at substantial odds with our results; consequently, it seems worthwhile to briefly review those literature values and note facts that support our results. Perhaps the most common value cited for the density of monoolein is 0.94 g cm⁻³ (molecular volume of 630 Å³) at 20 °C. The source for this value has been given as Sigma Aldrich, and as the CRC (Chemical Rubber Company) handbook, with both sources giving the same value. As the CRC value for monoolein has been listed for at least 40 years, it is likely the source used by Sigma Aldrich. A more recent measurement yielded 0.96 g cm⁻³ (617 Å³) at 37 °C; in that work, no mention of the other literature value was made. That measurement used a DMA 60 and a DMA 602 densitometer (Anton-Paar), which are both older, less accurate predecessors of the instrument used in this work. There are no explicit experimental accounts of whether or not the monoolein was hydrated in either of the measurements in the literature and so it is unknown as to the role hydration plays in the difference in densities. Nonetheless, it is noted that the higher temperature measurement has a higher density which does not seem likely.

In order to evaluate these densities, it is useful to consider the volumes and corresponding electron densities of monoolein’s components. At 25 °C, the terminal methyl occupies 56 Å³ and the remainder of the tail (excluding the carbon double bonded to the oxygen) occupies 413 Å³. Using the old CRC handbook value for the density and applying a slight expansion factor yields a volume of 631 Å³ for monoolein as a whole at 25 °C. Thus, the head group occupies 162 Å³ using the old CRC handbook value for the density of monoolein; the same calculation using our new results yields a head group volume of 129 Å³. Support for this dramatic difference can be found by considering monoacetin, which, chemically, is essentially monoolein’s head group plus a terminal methyl. The density of monoacetin is 1.21 g cm⁻³, as found in CRC-Handbook, its volume minus that of the terminal methyl yields 128 Å³, which is readily compatible with our results and in disagreement with the old literature values. Another item of support for our result can be found using ChemSpider, a freely available online chemical database owned by the Royal Society of Chemistry. Using ACD/Labs software, ChemSpider lists the predicted density of monoolein as 1.0 ± 0.1 g cm⁻³, which is compatible with our result and in conflict with the older literature values.

Also, our new results can resolve a puzzle in the literature by considering the electron density of the monoolein head group.
X-ray diffraction is sensitive to differences in electron densities. Phospholipids have head group electron densities of around 0.54 e Å\(^{-3}\) and water has an electron density of around 0.33 e Å\(^{-3}\); consequently the phospholipid head group has prominent, well defined peaks in electron density reconstructions utilizing X-ray data. There is also good agreement between simple strip models and those reconstructions.\(^6\) Using the old CRC handbook density results in an electron density of 0.39 e Å\(^{-3}\) for monoolein’s headgroup, which suggests that electron density reconstructions of monoolein will not result in well defined head group peaks. Indeed, modeling based on this value results in reconstructions in which artifact peaks are larger than the head group peaks. However, reconstructions of actual monoolein X-ray data result in well defined head group peaks and no major artifact peaks. To summarize, the phospholipid modeling and data matched well; the monoolein modeling and data did not match well.\(^6\) Using our new measurements results in an electron density of 0.49 e Å\(^{-3}\) for monoolein’s head group density. This is comparable to the density for phospholipid head groups, which in turn suggests that electron density reconstructions for monoolein should result in well defined head-group peaks, which is indeed what is seen. We anticipate further exploring and exploiting this feature in future work.

### 3.3 DSC scans and kinetic phase diagram

Baseline corrected DSC scans are shown in Fig. 3 for monoolein in a 2 mol kg\(^{-1}\) solution of sucrose.

Enthalpies were quite small, of the order 1 mJ mg\(^{-1}\), as is typical for these systems.\(^{41}\) The enthalpies varied slightly with ramp rate and composition; the variations did not exhibit a discernible pattern. However, the locations and widths of the transitions were readily reproducible and yield useful results. As is generally the case, the phase transition location is shifted further as the temperature scan rate increases. An interesting feature of this system can readily be seen in that the cooling transitions are shifted much more than the heating transitions.

This asymmetry in the location of the cubic–H\(_{II}\) transition for monoolein and sucrose solutions is in marked contrast to the symmetry seen in the L\(_{a}\)–H\(_{II}\) phase transition observed in phospholipid and water systems.\(^{22,62}\) The equilibrium phase transition temperature (\(T_0\)) can be found by plotting the phase transition temperature seen on heating (\(T_{\text{heating}}\)) versus the temperature seen on cooling (\(T_{\text{cooling}}\)) and extrapolating to the point where the two are equal. In practice, this is done by making a linear fit and determining where that fit intersects with the \(T_{\text{heating}} = T_{\text{cooling}}\) line (see Fig. 4).\(^{22,62}\)

The phase transition temperature dependence on temperature ramp rate and sucrose concentration can be summarized with a kinetic phase diagram (see Fig. 5).

Let us define the heating hysteresis as \(\Delta T_{\text{heating}} = |T_{\text{heating}} - T_0|\) and the cooling hysteresis as \(\Delta T_{\text{cooling}} = |T_{\text{cooling}} - T_0|\). A general feature is that the amount of hysteresis increases greatly with sucrose concentration. From the diagram, there is clear asymmetry in the heating and cooling hysteresis for lower sucrose concentrations but the asymmetry is reduced at higher concentrations. For both heating and cooling, the hysteresis markedly increases with sucrose concentration. The equilibrium phase transition temperature is strongly affected, being dropped by 19.8 °C (mol kg\(^{-1}\))\(^{-1}\) of sucrose concentration. By comparison, the effect of sucrose on the cubic–H\(_{II}\) transition is typically twice as great as on the L\(_{a}\)–H\(_{II}\) transition.\(^{42}\) Pleasingly, the extrapolated equilibrium phase transition temperature for pure water exactly matches the literature value of 90 °C.\(^1\)

### 3.4 Transition width and FWHM

In addition to the location of the transition, one can also extract valuable information from the widths of the DSC scans. In keeping with previous kinetics work, the transition width \(\tau\) is...
defined as the time for the phase transition to go from 1/4 to 3/4 completed.\(^{22,62}\) However, for DSC data, it is commonplace to report the FWHM (full width, half maximum). These quantities can be straightforwardly related by the following calculation. Let us approximate the shape of the DSC trace as a Gaussian. Consequently, we define a dimensionless Gaussian that has been normalized so that the total area under the Gaussian is 1, namely

\[
g(t) = \frac{1}{\sqrt{\pi}} e^{-t^2}.
\]  

(1)

The FWHM is then

\[
\text{FWHM} = 2\sqrt{\ln 2}.
\]  

(2)

In a region centered on the Gaussian, ranging from \(-x\) to \(+x\), the area under the Gaussian is given by the error function

\[
\text{Erf}(x) = \int_{-x}^{+x} g(t)\,dt.
\]  

(3)

The normalization of the Gaussian can be economically stated as \(\text{Erf}(\infty) = 1\) and \(\text{Erf}(0)\) can be physically interpreted as the fraction of the phase transition that is completed in the interval from \(-x\) to \(+x\).

We can now find the transition width \(\tau\) that corresponds to half the transition being completed. Mathematically, this is

\[
\text{Erf}(\tau/2) = 1/2,
\]  

(4)

which can be numerically solved to yield

\[
\tau \approx 0.954.
\]  

(5)

Consequently, \(\tau\) can be readily determined from the FWHM by

\[
\tau \approx \frac{0.954}{2\sqrt{\ln 2}} \text{FWHM} \approx 0.573\text{FWHM}.
\]  

(6)

### 3.5 Hysteresis, width and Avrami analysis

The hysteresis is well fit by a power law (see Fig. 6) and so we can write

\[
\Delta T_{\text{cooling}} = |T_{\text{cooling}} - T_0| = x_c r^\beta,
\]  

(7)

where \(\Delta T_{\text{cooling}}\) is the cooling hysteresis, \(T_{\text{cooling}}\) is the phase transition temperature seen on cooling, \(T_0\) is the equilibrium phase transition temperature, \(r\) is the cooling rate, and \(x_c\) and \(\beta\) are fit constants. The heating hysteresis is defined in a similar fashion and utilizes the same exponent \(\beta\) from the fit of the cooling data. The cooling widths also follow power law behavior (see Fig. 7) with the same exponent \(\beta\), allowing us to state

\[
\tau_c = x_c r^\beta,
\]  

(8)
where $t_C$ is the width of the cooling transition and $a$ is a constant derived from a fit. A compilation of the fit constants can be found in Table 1.

For low sucrose concentrations, roughly twice the hysteresis is seen on cooling as opposed to heating; as sucrose concentration increases, this asymmetry is reduced (see Fig. 8). The exponent $b$, which governs both cooling and heating hysteresis and the width of the cooling hysteresis, decreases markedly with increasing sucrose concentration (see Fig. 8). In this plot of $b$, there seems to be a possible shift at around 1.5 to 2 mol kg$^{-1}$ sucrose concentration, which is where the cooling and heating asymmetry starts to be reduced. Interestingly, the heating widths don’t exhibit much of a sucrose concentration dependence and are clustered around the same values. As the sucrose concentration increases, so does the viscosity of the solution. The increased viscosity could very well affect the lipid kinetics and may be responsible for the aforementioned shift.

With hysteresis and transition width data in hand, we can now proceed with an Avrami analysis by calculating the Avrami exponent. At the most fundamental level, the Avrami exponent $n$ tells how the amount of the new phase depends on time at the very start of the phase transition. In brief, if $\chi$ is the fraction of the system that is in the new phase, $t$ is time and $n$ is the Avrami exponent, then

$$\chi \sim t^n \quad \text{where} \quad \chi \ll 1. \quad (9)$$

If the phase transition proceeds by nucleation and growth, one can connect the Avrami exponent to the dimensionality of the pathway of the phase transition and the nucleation rate by which the transition is initiated. If a transition proceeds from a fixed number of nucleation sites, the Avrami exponent matches the dimensionality of the phase transition pathway. To wit, if the new phase grows in a linear fashion from a nucleation site, the Avrami exponent is one; if the growth is planar, the Avrami exponent is two, etc. If instead there is a constant rate of spontaneous nucleation, the Avrami exponent is increased by one, so that linear growth results in an Avrami exponent of two, and so on. If the rate of nucleation increases over time, the Avrami exponent also rises. Basic Avrami theory deals with phase transitions initiated by a temperature jump (see Schultz$^{63}$ for the basics) and was extended to phase transitions driven by temperature ramps Ozawa.$^{64}$ For systems exhibiting power law behavior, it has been further refined and the following relation derived,$^{22}$

$$n = \left(\frac{\beta t}{\ln 2}\right)^{\frac{1}{b}}, \quad (10)$$
where $n$ is the Avrami exponent and $x$, $\beta$, $r$ and $\tau$ are as defined earlier.

When $\tau$ exhibits the same power law exponent as the hysteresis, as is the case on cooling, we can write $\tau = \alpha_r r^\beta$, and our expression for the Avrami exponent, $n$, simplifies to a ramp rate independent expression, namely,

$$ n = \frac{x_C \beta}{x_r \ln 2} $$  \hspace{1cm} (11)

A compilation of the ramp rate independent Avrami exponents seen on cooling can be found in Table 1. The exponents fall in the range from around 2 to 2.5. One possible interpretation is that the cooling transition propagates via a one dimensional process that has a nearly constant nucleation rate; another possible scenario is that the transition proceeds primarily from a fixed number of nucleation sites and propagates via a two dimensional process.

The situation on heating is a bit more complicated, as the Avrami exponent ranges from about 1 to 3 and depends on both ramp rate and sucrose concentration (see Table 2).

Two general trends can be observed; the Avrami exponent increases as the ramp rate is reduced and as the sucrose concentration is increased. There does appear to be a slight decrease in the Avrami exponent for slower ramp rates for the 0.5 mol kg$^{-1}$ sucrose sample; however, the hysteresis is quite small at this low concentration and so these values have a high uncertainty. A possible interpretation of the general trends is that the transition proceeds by a one dimensional process that is initiated both by a fixed number of nucleation sites and spontaneous nucleation sites that appear at an increasing rate over time. In this scenario, transitions that happen quickly should be dominated by fixed nucleation sites and yield an Avrami exponent close to one; transitions that happen slowly would allow for greater contributions from spontaneous nucleation sites, resulting in a larger Avrami exponent. As both reducing the ramp rate and increasing the sucrose concentration result in an increase in the time the phase transition takes, this picture is consistent with the general trends we observe.

Besides classical nucleation and growth scenarios, it is also worth considering a non-classical picture. Because the cubic phase is so deeply perforated, it could very well be that the timing of the transition to this phase is dominated by pore formation; in this case, the cooling Avrami exponent reflects the time dependence of the pore formation. Likewise, the transition from the cubic phase to the HII phase could be dominated by pore absorption or repair and that the heating Avrami exponents reflect this process.

### 3.6 Structure

It is important to consider the geometry of structures involved in the phase transition (see Fig. 1). Key parameters are the water weight fractions and lattice parameters for the cubic and HII phases and the density of monoolein, which are summarized in Table 3.

For the following calculations, $V_L$ is the volume of monoolein, $\rho_L$ is the density of monoolein, $\rho_w$ is the density of water,
The area per lipid can be calculated via

\[ A_L = \frac{4\pi r_w \lambda}{\sqrt{3}a^2(1 - \phi_w)} \]  

The average lipid length requires a bit more consideration, as there is substantial variation of the length in the hexagonal phase. It has been shown that a volume weighted average\(^7\) can be found to a high precision by

\[ l = \left( \frac{1}{n} - r_w \right) \left[ 1.1084 + 0.0572 \left( \frac{r_w}{\frac{1}{2}n - 1} \right) \right]. \]  

The results of all of these dimensional calculations are contained in Table 3. Using these results, we can see monoolein’s length contracts by about 4% and the head group area decreases by 12% in the cubic–H\(_{II}\) transition.

As a comparison, one can consider the phase transition dimensions for rac-di-12:0 β-GlcDAG.\(^6^5\) As expected, there is hysteresis and the phase diagrams seen on heating and cooling are different. The data in the paper is from the cooling diagram and the H\(_{II}\)-cubic transition is about 70 °C for rac-di-12:0 β-GlcDAG. At that point, the lipid length in the cubic phase is about 15.8 Å. In the H\(_{II}\) phase, \(r_w\) is 16.0 Å and \(a/2 - r_w\) is about 13.5 Å, which results in an average lipid length of 15.1 Å. The average lipid length shrinks by about 5%. The area per volume, \(A_L/V_L\), is 0.051 Å\(^2\) in the cubic phase and 0.045 Å\(^2\) in the H\(_{II}\) phase. As the volume change between fluid phases is a good deal less than 1%,\(^31\) we will assume a roughly constant volume across the transition. This results in a reduction of the area by about 12%. Given that rac-di-12:0 β-GlcDAG is a lipid with two saturated tails 12 carbons long and that monoolein has a single monounsaturated tail that is 18 carbons long, the close correspondence of these changes is quite intriguing.

Another interesting comparison can be made to DOPE’s (dioleoyl phosphatidylethanolamine) L\(_c\)–H\(_{II}\) transition. DOPE’s two tails have the same structure as monoolein’s single tail. During the transition, DOPE’s length contracts by about 10% and the head group area decreases by about 22%.\(^7\) Thermodynamically, the cubic phase is intermediate between the L\(_c\) and the H\(_{II}\) phase, which makes sense given that the structure has curvatures that vary from flat to highly curved. Consequently, it also makes sense that these changes in lipid length and head group area are intermediate, being about half of those seen in the L\(_c\)–H\(_{II}\) system. Now, there are substantial differences between these systems; they have different head groups, numbers of tails and the transitions are separated by almost 90 °C. The fact that these systems can none the less be sensibly correlated suggests that these structural changes might well be of deep and broadly applicable significance.

### 3.7 Thermodynamics

A useful relation of the Clausius–Clapeyron type that governs the shift of the phase transition temperature of a system under the influence of a bulk solution has been derived\(^4^2\) and is

\[ \frac{dT}{dc} = \frac{k_B T^2}{Q} \left[ \Delta N_w - \frac{1}{c} \Delta N_w \right], \]
where $T$ is the phase transition temperature, $k_B$ is Boltzmann’s constant, $c$ is the bulk concentration of the solute, $Q$ is the latent heat of the transition, $\Delta N_w$ is the change in the number of water molecules going from the lower to the higher temperature phase and $\Delta N_c$ is likewise the change in the number of solute molecules. In our case, the solute is sucrose. It is also noted in passing that $c$ is best expressed as a unitless mole ratio; so a $c$ of 1 mole of sucrose per kg of water would be 1 mole of sucrose per 55.5 moles of water. Likewise, a proper normalization should be followed; we prefer to consider $Q$, $\Delta N_w$ and $\Delta N_c$ as per lipid quantities.

The quantity $\frac{1}{c} \Delta N_c$ is the change in the number of water molecules required to match the concentration in bulk; if the actual change in water molecules, $\Delta N_w$, is different from this, that difference is the change in the number of excluding waters. These excluding waters are not available to hydrate the solute and so are part of the hydration shell. This can be simply written as

$$\Delta N_w^{ex} = \Delta N_w - \frac{1}{c} \Delta N_c, \quad (20)$$

where $\Delta N_w^{ex}$ is the change in the number of excluding waters. Combining these two equations and solving for the change in excluding waters yields

$$\Delta N_w^{ex} = \frac{dT}{dc} \left( \frac{Q}{k_B T^2} \right) = -0.3 \text{ waters per lipid} \quad (21)$$

This value does not tell us the number of excluding waters in phase; however, we can use it to evaluate the plausibility of a variety of scenarios. The simplest scenario to consider is that of no excluding water in either phase; this would result in a $\frac{dT}{dc} = 0$ and so this case can be ruled out. In scenario that the sucrose is entirely excluded in one phase and there are no excluding waters in the other phase, the magnitude of the change in the number of excluding waters would be equal to the waters per lipid in the phase in which sucrose is excluded. This is therefore likewise ruled out as the size of $\Delta N_w^{ex}$ would be off by over an order of magnitude. Next, one considers the case where all of the waters in phases are excluding waters; this results in a $\Delta N_w^{ex} = -0.7$, which is of the right magnitude, but still off by a factor of two. This possibility also seems remote given the relatively commodious size of the water channels compared to the dimensions of a sucrose molecule. A final possibility to consider is that there is a hydration shell of thickness $t$ extending from each of the lipid head groups. By simple geometric approximation, we would expect

$$\Delta N_w^{ex} = \frac{(A_{hex} - A_{cubic}) t}{V_w}, \quad (22)$$

where $A_{hex}$ and $A_{cubic}$ are, respectively, the head group areas in the $H_{II}$ phase and cubic phase and $V_w$ is the volume of a water molecule, which is taken to be $30 \text{ Å}^3$. If the hydration shell is about 2.5 Å thick, of order of a single layer of water molecules, this would correspond to a $\Delta N_w^{ex} = -0.3$, which pleasingly matches our experimental result.

4 Conclusion

Sucrose has a powerful effect on the cubic–$H_{II}$ phase transition of monoolein, dropping the phase transition temperature and greatly increasing the hysteresis. Control of the phase transition is a useful tool for monoolein’s many uses, including drug delivery systems. Likewise, this study offers a useful base for further probing this transition. Control of the duration of the transition allows one to pick a time scale most favorable for the technique one wishes to use. Effects of other solutes can be examined to determine what effects are compound specific and which are more general features. A classical Avrami analysis suggests the heating cubic–$H_{II}$ transition proceeds via a one dimensional pathway and that the cooling $H_{II}$-cubic transition occurs via a one or two dimensional pathway. A non-classical picture is that the transition is wholly dominated by pore formation and destruction, with the Avrami exponents revealing the time dependence of these processes. Future work could include detailed exploration of either of these pictures, as well as efforts to deconvolute temperature ramp rate data into an isothermal phase transition model.

Correct values for the temperature dependent density of monoolein are essential to properly understanding the structure of these phases; preliminary calculations indicate that this new data will have a profound effect on the electron densities used in modeling X-ray diffraction data. We also show that the lipid length decreases by about 4% and that the head group area decreases by 12%, changes that match closely with a dissimilar lipid (rac-di-12:0 β-GlcDAG) in the same transition. These changes are about half that seen in the $L_{cubic}-H_{II}$ transition, which can be rationalized by recognizing the intermediate location of the cubic phase between the $L_{cubic}$ and $H_{II}$ phases. Structural work combined with thermodynamic insights suggests that there is a roughly one water layer thick hydration shell for monoolein. Much can be gained from thorough characterization of a system; besides contributing to a better understanding of monoolein’s phase behavior, it is hoped that the reader has a renewed appreciation for fundamental quantities such as lipid volume and the value of a structural perspective based on these fundamental quantities.

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