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Lipid Composition in Miscible and Immiscible Phases

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1. Introduction

Lipids play a vital role in the architecture of cell membranes. About one third of the known cellular proteins are located in the membrane lipid and these are largely the transporters, signalers, receptors and defense systems. The fatty acid component in membranes across species and in different cell types is species, organ and sub cellular specific. In human cell types, the inner cell membrane is dominated by arachidonic acid whilst that of the photo receptor and neural synapse is strikingly rich in docosahexaenoic acid. The biophysical basis for this molecular specificity in not understood.

Differences in lipid composition and lipids membrane packing must inevitably affect cell function. Dietary deficiency of docosahexaenoic acid (DHA) in pregnant rats has been shown to reduce fetal neurogenesis and neuronal cell migration [19]. Deficiency in infants can restrict visual and cognitive development [18]. Close structural analogs of DHA do not displace DHA from active sites even when such analogs are present at higher concentrations than DHA in the diet or in cell systems [3]. Thus the chemical structure of lipids at active sites is conserved at the molecular level by dietary lipids but lipids generated biochemically somehow do not displace or dilute them.

NMR spectroscopy, X-ray crystallography, and computational chemistry are essential tools in discriminating the molecular shape of biomolecules. Although NMR experiments using Deuterium-Labeled NMR have long been used to probe phospholipid bilayers [17], far fewer experiments yielding structural information have been generated for lipids than for proteins and carbohydrates. Natural abundance ^{13}C-NMR experiments with DHA demonstrated that highly unsaturated lipids pack more closely than more saturated lipids [2]. However, identifying the specific inter-molecular sites critical to lipid packing was not discernable.

Discrete chemical compositions of mixtures at the molecular level within definable unit cell molar volumes have been reported for stearic acid (SA) in the presence of structurally

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different solvents [13]. Although above a specific concentration, SA was "insoluble" in methyl oleate (MeOA); the immiscible phase was not SA, but a more densely packed MeOA/SA phase [14]. We report herein that an immiscible lipid phase on adding SA also occurs with structurally diverse lipids (e.g. DHA, pig fat and seal oil) and the thermal properties of the second phase are a fingerprint marker of its initial lipid identity. Differential scanning calorimetry (DSC) experiments distinguish the kinetic components from the thermodynamic components of these lipid physical properties.

2. Materials and methods

2.1 Materials

Stearic acid (SA) and methyl oleate (MeOA) were obtained from Sigma Aldrich (St. Louis, MO, USA). Pig fat and seal oil were provided by Alva Mitchell (ARS, Beltsville, MD) and fish DHA and DHA-PC samples were provided by Michael Crawford (UK).

2.2 Differential Scanning Calorimetry (DSC)

DSC measurements were performed using TA Instrument's Q200 calorimeter and a universal 4.5 data analysis software from TA Instrument. Approximately 15-20 mg of lipid samples were sealed in aluminum DSC pans and subjected to either traditional DSC or modulated DSC (MDSC) analysis. A sealed empty pan was used as reference. For DSC analysis, the sample was equilibrated at 65°C and kept isothermally for 30 minutes at that temperature and then cooled to 25°C at a rate of 1°C min-1. The sample was then heated to 65°C at the same rate, and this cycle was repeated for 3 more times. For modulated DSC, the experiment lasted for 10 hrs. The sample was cooled down stepwise by initially equilibrating it at 65°C and the temperature was modulated at the rate of +/-2°C every 200 sec. The first cooling step was 48°C and then it was cooled down by 2°C and this process continued until the temperature reached 40°C. At each step the sample was kept in isothermal condition for 100 minutes.

2.3 Raman spectroscopy and imaging microscopy

Samples (ca. 1–10mg) were placed on an aluminum tape lined glass slide. Raman spectra were collected using a LabRam Aramis confocal Raman microscope (Horiba Jobin Yvon, Edison, New Jersey) with a 10×, 50×, and 100× objectives. The spectra were collected over the range of 200–4000cm−1 Raman shift using a HeNe laser (633 nm) for excitation. The confocal hole, and slit aperture were 500_m and 100_m, respectively. Maps were generated using a grating of 1200 g/mm, exposure time of 5 s with 20 accumulations per data point. In maps, spectral range was reduced to 600– 1800 cm−1. For Raman imaging, and map of mixtures, a microscopic field of view was selected to include two lipid phases simultaneously. In map processing of mixtures following frequency ranges were selected: 1035–1162 cm−1 (for C C group), 1403–1459cm−1 (for C H group), and 1476–1772 cm−1 (for C C and C O group). On the region chosen for mapping, the program plots the localized distribution and intensity of these specific peaks.

2.4 Molecular mechanics

The chemical structure of individual lipid molecules was generated on HyperChem 8.0 (Gainesville, FL). The packing among lipid molecules was optimized using MM+ force fields

(Zhdanov et al., 2003). The conjugate gradient (Fletcher-Reeves) method was used to energy minimize distances among lipid molecules. Convergence condition limits were a RMS gradient of 0.01 kcal/Å mol. The packing among the lipids was the smallest cubical molecular volume in which stoichiometry could maintain the macroscopic mole ratios determined experimentally.

3. Results and discussion

3.1 DSC

The thermodynamic component of miscibility and immiscibility has not previously been addressed. The DSC curves of structurally different lipids (MeOA, DHA, seal oil, pig fat), cycling through the temperatures 25°C to 65 °C four times, are presented (Figure 1a). Once a threshold concentration of SA was added to MeOA, the DSC curve showed a readily distinguishable phase transition in each cycle in which the lipid becomes cloudy (Figure 1b). MeOA was also the major component in the second phase as confirmed by Raman Spectroscopy [14]. The SA concentration in the clear phase remained identical to that of the threshold concentration and the higher concentration of SA occurred only in the second phase.

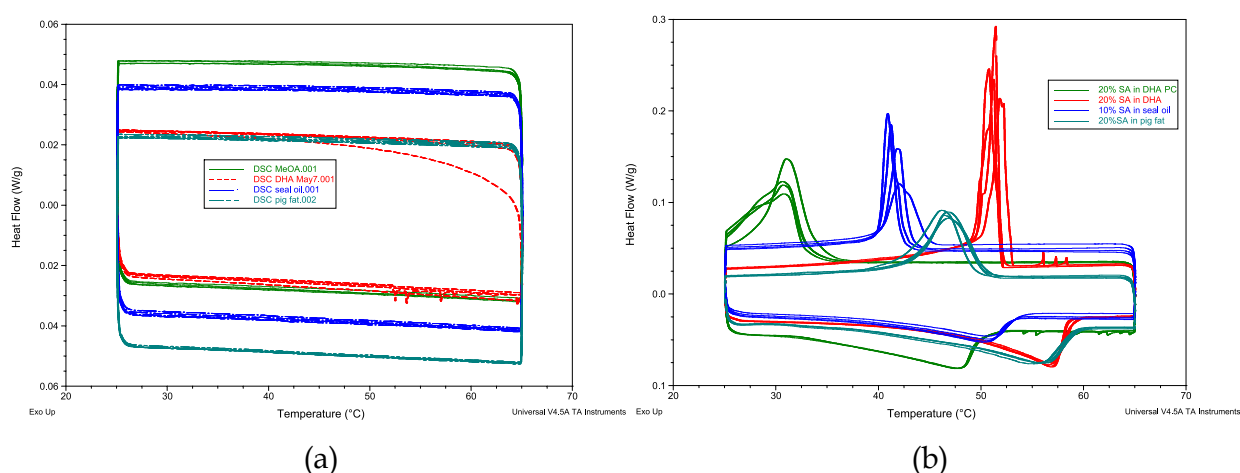(a)   (b)

Fig. 1. (a) The natural lipids have no identifiable phase transition in the Differential Scanning Calorimetry (DSC) curves. (b) Discretely different phase transitions of same lipids upon including 10-20% Stearic acid (SA).

Lipids are assumed amorphous because thermal properties of miscible lipid phases are unaffected by chemical structure. Differential scanning calorimetry (DSC) (Figure 1a) of methyl oleate (MeOA), docosahexaenoic acid (DHA), pig fat and seal oil are presented. Heating and cooling cycles were repeated four times yielding minimal thermal information. Phase transitions were absent likewise for high purity single component fatty acid methyl ester (MeOA) as for a complex mixture of natural triacylglycerides (seal oil). Mixtures of these lipids with 5% stearic acid (SA) remained fully miscible and no phase transition is observed. DSC of SA in DHA-phosphotidylcholine (DHA-PC), seal oil, pig fat, and DHA (Figure 2b) demonstrates an immiscible phase forms, and thermal properties among structurally different lipids are discretely different from each other.

A cube can have the average length of a lipid molecule (e.g. MeOA). The lipid composition in that space can have a same stoichiometry at the molecular level as is determined at the

macroscopic level. Four rows of five MeOA molecules are the most that can fit into a cubic volume. Since 5% SA equals 1/20, the structurally simplest molar volume at this mole ratio contains 21 molecules (Figure 2). For comparison, the same unit cell can be oriented such that the single SA molecule is in an X, Y, or Z direction. The cross sectional area of each of the surfaces is similar. Computational chemistry optimized the packing and the spacing within and between cubic unit cell volumes of adjacent lipid molecules and adjacent unit cells, i.e. van der Waals distances from each other.
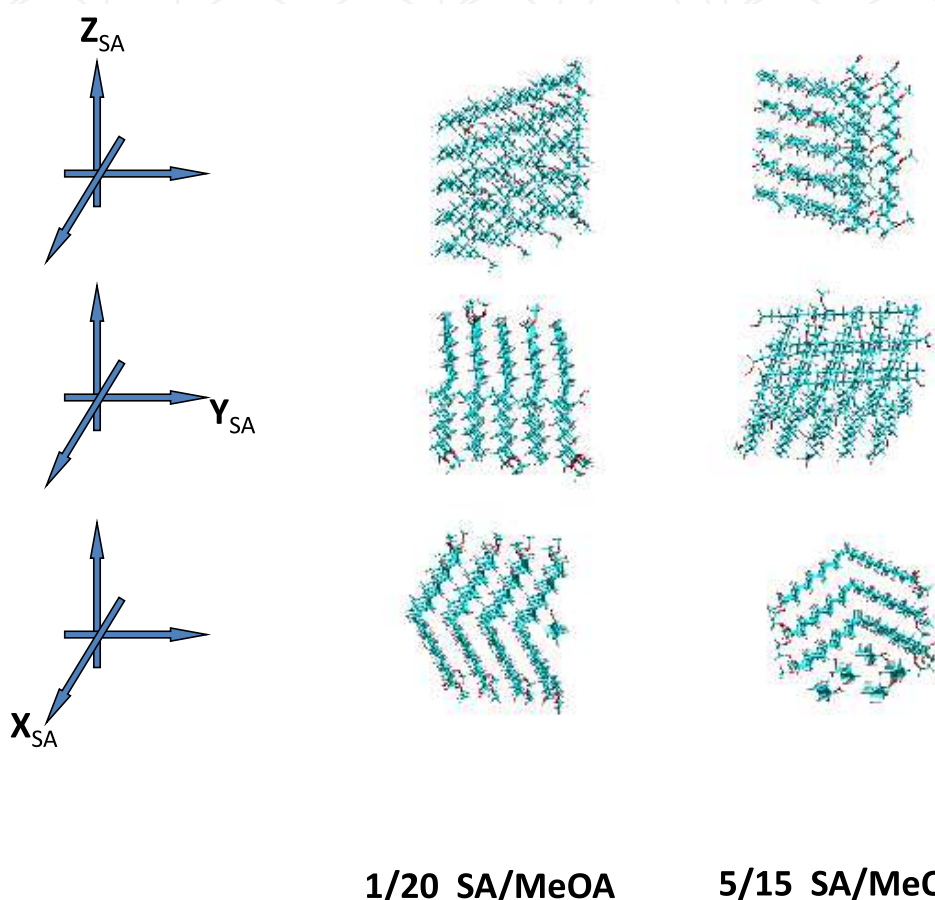

1/20 SA/MeOA   5/15 SA/MeOA

Fig. 2. Stoichiometry in packing of SA lipid unit cell volumes of Methyl Oleate (MeOA) and Docosahexaenoic acid (DHA). DHA packs more efficiently in unit cell volume than MeOA.

A discrete lipid stoichiometry requires predicable and efficient packing. Three unit cell volumes are presented in 3-D views: Stearic Acid (SA) in Methyl Oleate (MeOA) (1/20), SA in MeOA (5/15), and SA in Docosahexaenoic Acid (DHA) (4/20). The Cartesian axes align with SA orientation within the cubic unit cell volume (views = X_{SA}, Y_{SA} and Z_{SA}). SA enhances the packing density of lipids within the unit cell volumes.

Lipid packing is a barrier to the flow of lipids from unit cell to unit cell and also into/out of aggregates of lipids. Steric hindrance precludes aggregates from unpacking. Void volume is the space that enables the mobility of lipids within the unit cell volume. Mathematically, the void volume in optimally packed equally sized spheres is 26% of the total volume; poorly packed spheres have up to 34% of the total volume empty [1]. In contrast void volume within a cubic unit cell can be 10% of the total volume.

Once a SA molecule filled the void volume in the lipid unit cell volume, diffusion into neighboring unit cells would be inhibited. By increasing the concentration of SA, the only structural space available would be from packing existing unit cells more efficiently. A 1/3 = 5/15 ratio of SA/MeOA packed efficiently into unit cells (Figure 2a). The mass of the unit cells contained 20 lipids whereas the 1/20 unit cells contained 21 lipids, so the kinetic energy of the 5/15 lipid had 5% less mass per unit cell than the 1/20 unit cell volume lipid. The kinetic energy of the smaller unit cell volume at a given temperature will be about 5% faster than the larger one. When a unit cell is anisotropic, the molecular dynamics will be anisotropic. X_{SA}, Y_{SA} and Z_{SA} directions label the axis corresponding to the axis of the SA molecules in the unit cell volume. In the X_{SA} direction, the unit cell was more tightly packed in the 5/15 ratio than in the 1/20 ratio unit cell; in the other two dimensions, the packing differences were almost indistinguishable from each other.

For mixing to occur, unit cell volumes need to exchange places with other lipid unit cell volumes (Figure 3). The void volume between unit cells was largest in the X_{SA} direction in the 5/15 unit cell. Mixing occurs with the neighboring unit cell in which X_{SA} direction of both are aligned. In this proposed mechanism, during one clockwise rotation of the 5/15 unit cell volume, five molecules of MeOA and 4 molecule of SA can very efficiently exchange site with a neighboring 1/20 unit cell volume. Thus, the 5/15 unit cell moved 25 Å (the length of a unit cell) in a Y direction and the 1/20 unit cell moved 25 Å in a (–Y) direction. Rotational motion resulted in translational motion.
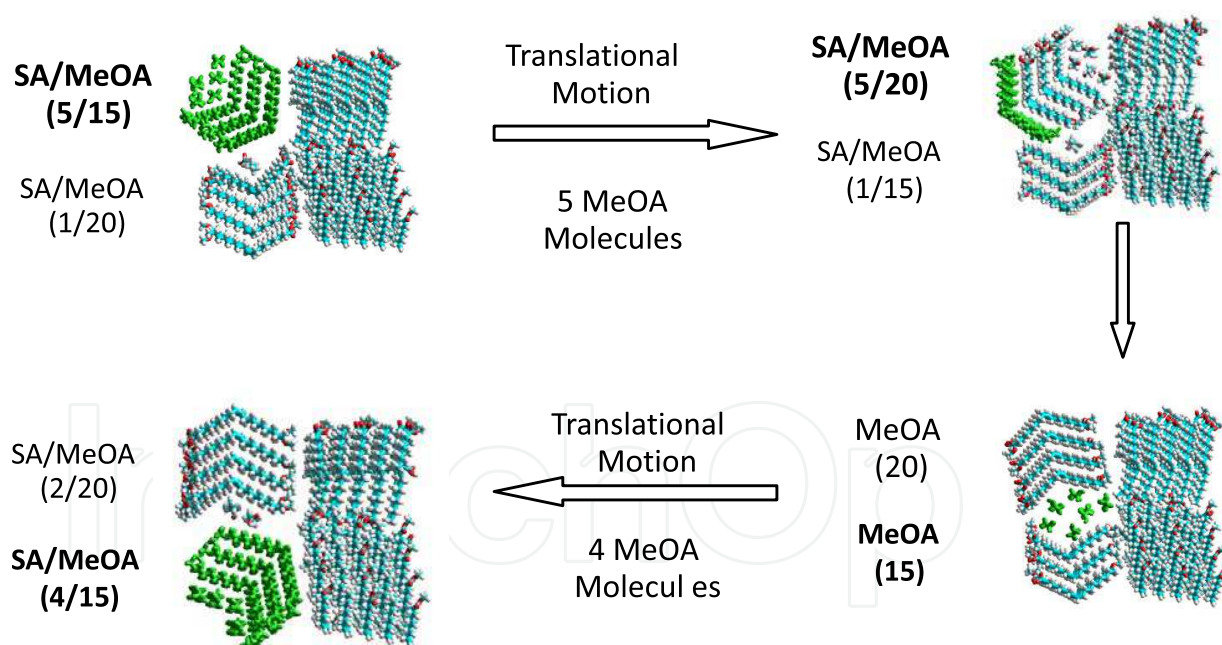

Fig. 3. Proposed mixing between two Unit Cell Volumes, each with a different lipid composition stoichiometry.

Lipid translational motion requires sufficient empty space/volume for this to occur. When three unit cell volumes of (1/20) mix with one unit cell volume of (5/15) SA/MeOA, the more dense packing in the (5/15) unit cell creates the space for SA exchange between two neighboring unit cells. Mixing of lipids between unit cells can appear to be diffusion of unit cell volumes, when in fact only a fraction of the total lipid molecules would be moving.

Uniformity in the distribution of unit cell volumes is not the same as uniformity of lipid composition "unit cell to unit cell." Translational motion of a specific chemical composition between two unit cells enables dispersing the unit cell with the higher SA concentration throughout the lipid. Lipids may be composed of at least two distinct unit cell volumes, non-uniform at the molecular level, yet macroscopically fully interspersed/mixed. Alternatively, lipids immiscible at the molecular level can also be non-uniformly dispersed throughout the lipid.

The DSC detected phase transitions during both cooling and heating cycles (Figure 4). On cooling the 2/20 SA/MeOA mixture from 65°C to 51°C, no thermal evidence of a phase transition was found. A very sharp DSC phase transition (width: 2 °C) began at 48 °C on the second cycle and the temperature lowered to 45 °C by the fourth cycle. A rapid exothermal phase transition indicates the products of this phase transitions were rather uniform, and suggests the mechanism causing it is uncomplicated. The simplest interpretation of the data is: two phases formed two discrete uniform unit cell volumes from one single uniform lipid liquid phase. The single uniform unit cell volumes could for example be same size and shape as the 5/15 unit cell volume. However, because of stoichiometry, for every 1.5 molecules of SA/15 MeOA present, the volume of 3.5 molecules of SA had been replaced with void volumes (1.5 +3.5 = 5). Alternatively, the unit cell volume could also be some volume intermediate between the two. Void volume adds to the average unit cell volume, and adds nothing to its mass. The sudden loss of volume with no loss in mass most probably is the structural basis for the rapid phase transition.
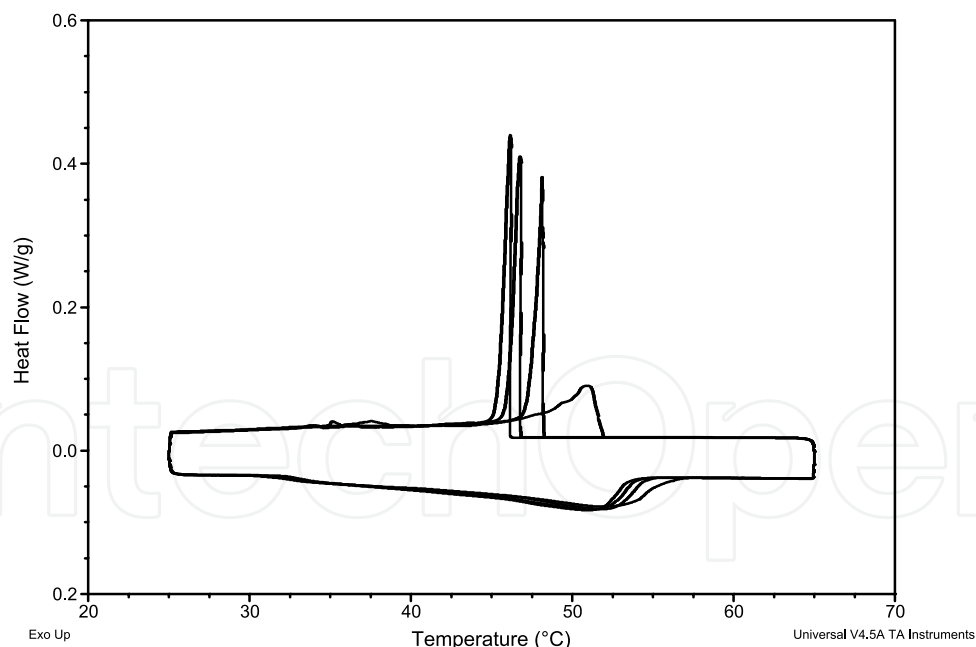

Fig. 4. DSC of two component lipids: SA and MeOA. Cycling of temperature enhances solids properties and uniformity of solids properties.

DSC of SA in MeOA (1/10). At 10% SA (wt/wt), the mixture with MeOA turned cloudy and a second phased forms. On cooling, a stable immiscible phase SA/MeOA (5/15) rapidly formed. Repeated cycling sharpened the phase transition on cooling but the very broad curve on heating remains quite similar.

In contrast, the very broad endothermic phase transition on heating occurred over a 20 °C temperature range. Mixing on heating took place by a different mechanism than separating out on cooling. Incorporating void volume into lipids is required for significant mixing to occur. Thus, the distribution of chemical composition and void volume during heating is necessarily localized. Since unit cell volume with different compositions have different mass, and unit cells with the same mass incorporating different void volumes will have a different unit cell volume, the rate of absorption of heat will not occur uniformly unit cell to unit cell. The unit cell volume, void volume and chemical composition must each be spatially uniform before the equal thermal properties at the molecular level can be spatially uniform.

Identifying fish oils by differences in thermal properties using DSC at very rapid heating and cooling rates has been reported [15]. Very rapid temperature ramping produced sharp phase transitions not evident in regular DSC experiments (as in Figure 1d). Rapid ramping can hide the asymmetry of fast cooling–slow heating kinetics, thus making both rates appear equal and the processes seem to be symmetrical.

Recently, modulated DSC (MDSC) procedures were developed [12]. Heat is added sinosoidally in place of linearly ramping temperature ($\Delta H_{mix} = 0$). MDSC can enable the thermodynamic and kinetic properties to be distinguished.

The temperature independent component (ΔS_{mix}) of the free energy of mixing (ΔG_{mix}) is accessible from constant temperature experiments. Thus the MDSC experiment was applied to 1/10 SA/MeOA (Figure 5). With a modulated heating rate and the sample held at a constant temperature for 100 minutes in each step (48 °C to 40 °C), changes were monitored. A very slight change in slope of the heat capacity (C_p) curves was observed for each temperature step until the sample reached 40 °C at which Cp increased by 23%. The thermal measurement was 5 – 10 °C lower than the phase transition temperature in the DSC experiment. Thus the DSC results included kinetic as well as thermodynamic components.

The MDSC result supports the conclusion that lipids do not necessarily transfer heat rapidly or uniformly. Thus some sites at the molecular level could be cooled to 40 °C even if the net temperature of the lipid was 48 °C. The change in Cp at 40 °C corresponds to the rapid conversion of a one phase-one unit cell volume into a two phase–two unit cell volume mixture in which each phase has a specific stoichiometry. In MDSC, solids properties form "quickly" in 100 minutes. The similar solids properties can be obtained from the same lipids in non-uniform temperatures in over 7000 minutes. Once formed, however, the solids properties last indefinitely. Latent heat in lipids may dissipate slowly because time is required for stoichiometry to be uniform enough for "relaxation" to occur. A simple mechanism to explain latent heat is that unit cells too large or too small to pack uniformly will diffuse into localized pockets of similar sized pockets. An equally viable explanation is that unit cell volumes containing variable amounts of packing inefficiency. Void volumes will migrate away from those which have less variability in packing volume. The unit cells which are more variable are most prone to absorb heat over a larger area; the areas which are most uniform in composition will heat and cool most uniformly. Non-uniformity in packing within a unit cell volume will most always correspond to non-uniform chemical composition within that unit volume.

The MDSC experiment corresponds to in effect an adiabatic constant temperature experiment. When the unit cell volumes are uniform, solids properties occur even when individual lipid molecules between unit cells may not be exactly uniformly packed. Even relatively small fluctuations in temperature may keep components mixed that at a constant temperature would in short order become immiscible.
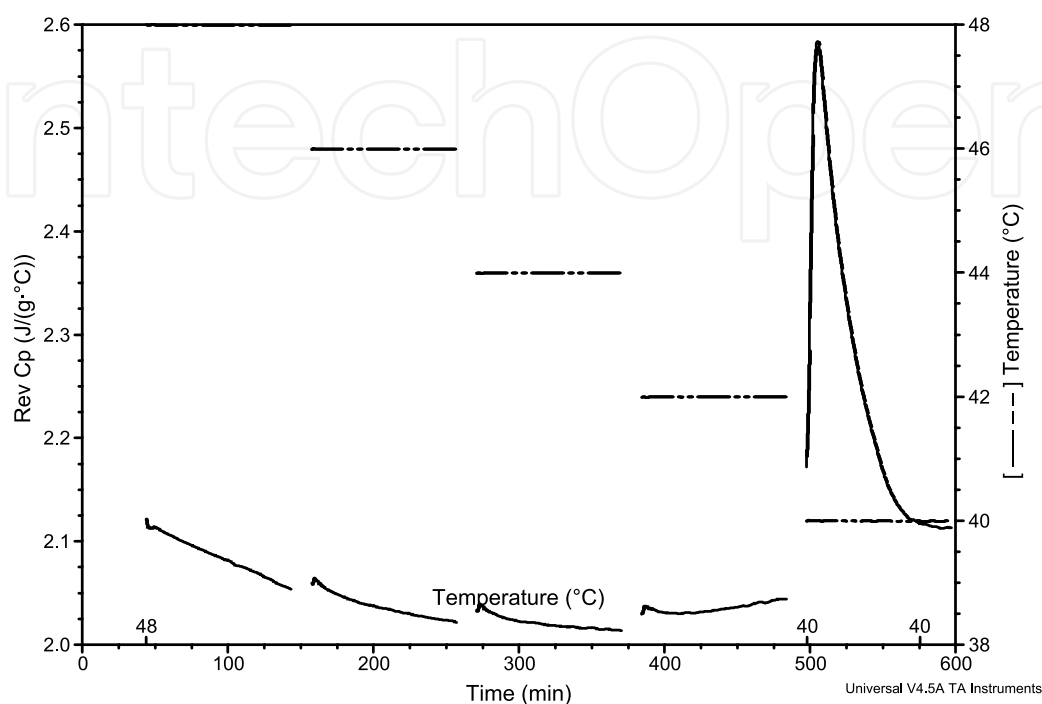

Fig. 5. Modulated DSC experiment in SA and MeOA demonstrates solids properties of lipid mixtures are thermodynamic properties. Kinetic properties of lipids can mask thermodynamic properties.

Although the precise chemical composition of and isolation procedures for natural lipids is variable, the molecular structure of DHA, seal oil, pig fat, and DHA-phosphotidylcholine (DHA-PC) has previously been characterized [2, 5, 6, 7, 9, 11]. Seal oil phase transition on cooling (42 °C) is easily distinguishable from the less unsaturated pig fat (51 °C). DHA phase transition (52 °C) is very different from DHA-PC (35 °C). The DSC of lipids (+ SA to form an immiscible phase) can be a rapid first pass tests for distinguishing whether two lipids are identical. This is evidence even though the SA portion in each has the identical size and shape, the 3-D packing around and within each unit cell volume depends upon the chemical composition of the non-SA lipids.

More important, however, is applying the thermal results to the molecular level packing of biochemically important lipids like DHA, lipids like the highly unsaturated DHA which dominates the composition of the photoreceptor, neurons, and synapses in the brain [3]. Ordered, close packing of the fatty acid chains in DHA neural lipids can facilitate the electrical functions (Crawford et al., 2008). DHA is a single chain lipid with six un-conjugated double bonds. SA + DHA had the DSC curve on cooling with the highest phase transition temperatures of the lipids analyzed. The shape of the DSC curve of DHA + SA was similar to that of MeOA + SA, except twice the level of SA was required to form an immiscible lipid phase and the phase transition temperature was higher.

The size and shape of a unit cell volume allows for only a specific number of molecules (each of which has its own size and shape). Especially large long molecules, inefficient packing always creates a large volume in which nothing large fits. The existence of stoichiometry in lipid unit cells is strong evidence that in fact unit cells are discrete sizes and shapes.

DHA molecular weight (328.5) is 111 % greater than for MeOA (296.5). Computational chemistry enabled comparing the packing in cubic unit cell volumes of immiscible phases containing (4/20) SA/DHA (volume = 14,484 A^3) with that of 2/20 SA/MeOA (volume = 13,128 A^3) (Figure 2a). The increase in mass was self consistent with the 110 % increase in volume, except the DHA volume contains four molecules of SA, not two as with MeOA. Thus with only one SA molecule per unit cell of DHA, a void volume equivalent to three molecules of SA would be present. Tight packing of the immiscible DHA phase at the molecular level can explain the high phase transition temperature of its DSC cooling curve. The thermodynamic data and the computational chemistry concur with the NMR study that previously reported that more highly unsaturated lipids pack tighter than less highly unsaturated lipids [13].

Interestingly, the molecular order within the unit cell parallels that of molecular order in lipid bilayer phases. Molecular level organization has previously been reported in lipid bilayers containing DHA [11]. They propose however molecular order in the bilayers is due to the presence of cholesterol. An alternative radically different explanation is that bilayer lipids already exist in unit cell volumes, and that cholesterol at the proper mole ratio fills up the void volume in the spacing of the lipids: in the presence of excess cholesterol, an immiscible phase forms. A similar mechanism can cause lipid bilayer formation. An unexpected conclusion of this research is that an ordered immiscible phase unit cell volume may routinely forms from an ordered miscible phase unit cell volume.

The miscibility/immiscibility phase separation in lipids is directly analogous to phase separation in ethyl acetate/water mixtures. Ethyl acetate freely dissolves in water up to a specific mole ratio; above that ratio, a second phase forms. The top layer is ethyl acetate saturated with water, the bottom layer is water saturated with ethyl acetate. As long as both phases are present, the further addition of ethyl acetate does not change the composition of either phase: only the volume fraction of each phase alters. The packing within the two distinct mole ratios of ethyl acetate to water results in a discrete phase difference. *There is no structural difference between the two layers because both layers contain exactly the same chemical components.* In the absence of a specific stoichiometry to the contrary, all lipids would be fully miscible. The simplicity of the experiments which result in lipid immiscibility is compelling evidence that the packing in the miscible phase and in the immiscible phase are not identical.

The immiscible phase in the case of SA/MeOA can be a soft solid, a gel, or even a liquid crystal. The immiscible phase is not a random event, but the direct consequence of a stoichiometry that exceeds the requirement of lipid miscibility. Moreover since it is the result of a thermodynamic process, once the immiscible phase forms, it stays around indefinitely.

A broad range of individual phase transition temperature and peak sharpness of DSC curves of in induced immiscible phase from only four structurally diverse lipids was found.

A very practical potential application of this technology is that by adding a small excess of SA (forming an immiscible phase) to a lipid, markers of biophysical characteristics of specific individual lipid compositions could be identified for quality control purposes or for distinguishing between natural and adulterated lipids.

Similar lipid phase transitions due to immiscibility most certainly occur routinely elsewhere. The difference between hard and soft bacon fat is primarily due to type of fat in the diet (i.e. saturated vs. unsaturated fat) [8]. Compositional difference between the two could be that soft bacon fat equals a miscible lipid phase and hard bacon equals an immiscible lipid phase. The primary difference between white and brown fat forming is the cell structure and tissue vascularization [16]. Brown fat can have systematically different physical properties from white fat in that white fat could have a different unit cell volume than brown fat. A diet rich in saturated and trans isomer fats is known to be a high risk factor for cardio-vascular disease [10]. These fats when present in the plasma membrane of the arterial endothelium could induce an accumulation of thicker, denser lipid in the membrane. This lipid phase immiscibility could explain the vasoconstriction causing the higher blood pressure required to force blood through smaller, less elastic capillaries. Trans fatty acids as well as saturated fats could similarly disrupt the packing of plasma membrane lipids. Exactly the same chemical components can be present below and above a critical triggering concentration ratio. In the immiscible phase, lipid mixtures packed more efficiently than precisely the same lipid components in a miscible phase: the fat in the newly formed phase would be more dense. All that changed chemically in forming a second phase would be a discrete change in the mole ratio of the new phase. The immiscible lipid phase would be thermodynamically stable.

Biodiesel mixtures of lipids are often initially perfectly clear. Days later, the same solutions turn cloudy. A simple explanation of cloudiness in biodiesel is that in some mixtures of biodiesel lipids, latent heat is released and some of the lipids pack more efficiently forming an immiscible phase. When latent heat is present, some units of volume with one mixture of lipid chemical structures retain more heat than per volume with a different ratio of lipid chemical structures. The molar volume over which chemical structure and thermal properties of lipids are integrated needs to be self-consistent. With spherical molecular volumes, summation of the global volume is larger than the volume of the spheres. A dam built of packed of soccer balls leaks because the volume of the dam is larger than the summation of the volumes of the soccer balls. The boundary between two discrete lipid phases is like a dam, slowing the mixing at the molecular level between two phases such that stoichiometry in each phase is discrete. The spherical model of lipid packing is "too leaky."

4. Conclusions

Structurally diverse lipids do not necessarily pack uniformly at the macroscopic or the molecular level. Since each of the mixtures of structurally diverse lipids had a finite capacity to incorporate long straight chain lipids [i.e. SA], macroscopic and/or molecular space had to have been available for the mixture of lipids to remain fully miscible. When this excess volume is filled [e.g. with SA], capacity for additional lipids of similar size/shape are precluded because the space has already been incorporated by the added lipid.

Additional SA does not perturb the miscible phase: in each of the lipid mixtures, Raman spectral frequencies are indistinguishable from of the same lipid phase before excess SA is added. The immiscible phase which forms unambiguously has more unsaturated lipid than saturated lipid. SA does not concentrate in an immiscible phase. Instead, it alters the packing of miscible phase such that the mixture is more dense. Adding additional SA does not alter the stoichiometry of the immiscible phase: it lower the mole fraction of the remaining miscible lipid phase. Unlike packing within a cubic unit cell volume, a spherical model of lipid packing neither predicts nor can explain a mechanism for this to occur.

Macroscopic stoichiometry occurs but it cannot explain any specific discrete molecular based stoichiometry. The smallest regularly shaped volume which adequately explains stoichiometry at the molecular level is the cubic unit cell volume. An immiscible phase perhaps may not be able to form without a new stoichiometry discretely different from the initial one.

DSC results demonstrate adding a small mole fraction of SA in structurally diverse lipids creates an ordered lipid phase. A spherical model of lipids predicts over time an ordered lipid state will diffuse into a uniform mixture. Instead, despite entropy, unit cells of similar size and shape form and then slowly pack uniformly, resulting in macroscopically observed lipid order that lasts indefinitely.

Stoichiometry in lipids is a normal consequence of molecular order at the unit cell level. Saturated lipids and trans-mono-unsaturated lipids are on average long straight rods; cis-mono-unsaturated lipids are at the molecular level are a flattened V shape; poly-cis-unsaturated lipids are a U shape. DHA is approximately an O shape. Mixtures of lipids of different shapes [and roughly the same molecular weight] appear to pack more efficiently than single component unsaturated fats alone. That more than one ratio of lipids can pack into a similar, if slightly smaller sized unit cell is the structural basis of immiscibility. The smallest volume in which the stoichiometry lipids can be uniform is a unit cell volume with one dimension being the molecular length of a single lipid molecule.

Since the physical properties of lipids correspond to the properties of aggregates of [e.g. 25] lipid molecule, mixtures of lipids of the same unit cell volume will always have physical properties similar to those of single component lipids to the extent that its unit cell volume is similar. Adding sufficient SA to a lipid alters the unit cell volume which alters its apparent density which in turn creates the macroscopically observed immiscible phase.

The stoichiometry between two immiscible phases will always be different.

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Stoichiometry and Research - The Importance of Quantity in Biomedicine

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The aim of this book is to provide an overview of the importance of stoichiometry in the biomedical field. It proposes a collection of selected research articles and reviews which provide up-to-date information related to stoichiometry at various levels. The first section deals with host-guest chemistry, focusing on selected calixarenes, cyclodextrins and crown ethers derivatives. In the second and third sections the book presents some issues concerning stoichiometry of metal complexes and lipids and polymers architecture. The fourth section aims to clarify the role of stoichiometry in the determination of protein interactions, while in the fifth section some selected experimental techniques applied to specific systems are introduced. The last section of the book is an attempt at showing some interesting connections between biomedicine and the environment, introducing the concept of biological stoichiometry. On this basis, the present volume would definitely be an ideal source of scientific information to researchers and scientists involved in biomedicine, biochemistry and other areas involving stoichiometry evaluation.

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