Lateral chain packing in lipids and membranes

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INTRODUCTION

The wide range of fatty acid structures that occur in the complex lipids of membranes, lipoproteins, and lipid deposits within cells or tissues create well-known problems with respect to aliphatic chain packing. While it has generally been accepted that membranes, cores of lipoproteins, and lipid deposits in tissues are in the liquid state, it is possible that certain domains may at times be solid. If lipids, which contain a large variety of aliphatic chains, can undergo transitions from liquid to solid and solid to liquid, what is the nature of their chaining packing, particularly in the solid states?

The great diversity of aliphatic chains found in membrane and other lipids is illustrated in **Fig. 1,** which shows space-filling models of five fatty acids of increasing unsaturation. The configurations of these fatty acids conform to the crystalline structures determined for saturated long-chain fatty acids (1), oleic acid (2), and linoleic, linolenic, and arachidonic acid (3). Clearly, the conformation of these molecules and the specific crystalline structures determined for each of them are different. All of the saturated fatty acids form an elongated, nearly straight chain with angles between the carbons of approximately 110°, whereas oleic acid forms a sharp kink at the double bond, redirecting the chain at an angle away from the double bond. The remaining three molecules tend to maintain their elongated form by rotating the position of the double bonds in such a way that each *cis* double bond is in a *trans* position relative to the next double bond in the chain. Clearly, interactions between the saturated and unsaturated fatty acids, or between the different unsaturated forms, will present steric problems unless new conformations are taken in the chains. This review summarizes the specific kinds of chain packing that can occur in molecules of single-chain species; it considers the interaction of two similar molecules whose chains differ to see whether there is interaction in the solid state; and it examines the chain packing of certain molecules in which different chains are connected to one another through covalent bonds, for instance, in diglycerides, triglycerides, and phospholipids. Lastly, some inferences are made about chain packing in the biological state.

PACKING OF PURE HYDROCARBON CHAINS

The hydrocarbon chain in its most rigid, stable state forms a zig-zag, all *trans* arrangement from carbon to carbon (Fig. 1, see stearic acid). The distance between carbons is about 1.533 Å, similar to the carbon-carbon distance in the diamond. The angle between carbons varies slightly but is about 109°–112°. Thus, the distance between every other carbon is 2.54 Å, and the increment along the chain for each carbon is 1.27 Å. In the various crystalline packings of chains, the angles and distances vary rather little from atom to atom (4).

Crystalline alkanes and hydrocarbon chains of substituted alkanes pack in two quite distinct classes of subcells depending upon the lipid, the method of crystallization, and the temperature and pressure. The first class is characterized by dense, tightly packed chains in which there is *specific* chain-chain interaction. In the second, the chains are more loosely packed, and specific chain-chain interaction is lost due to partial local rotations along the chain. By viewing a plane of the hydrocarbon chains perpendicular to their axes, a two-dimensional lattice can be described. Of the four types of two-dimensional lattices, square, rectangular, oblique, and hexagonal, hydrocarbon chains can pack in the last three. When the hydrocarbon chains form specific chain-chain packing, they pack into either oblique or rectangular lattices. With nonspecific crystalline packing, the chains pack in a true hexagonal lattice (see Fig. 2). The nonspecific chain packing lattice may also be a two-dimensional rectangular lattice which approaches hexagonal symmetry. The nomenclature and characteristics of the different chain packing modes are summarized in **Table 1** (5).

Abbreviations: DSC, differential scanning calorimetry.

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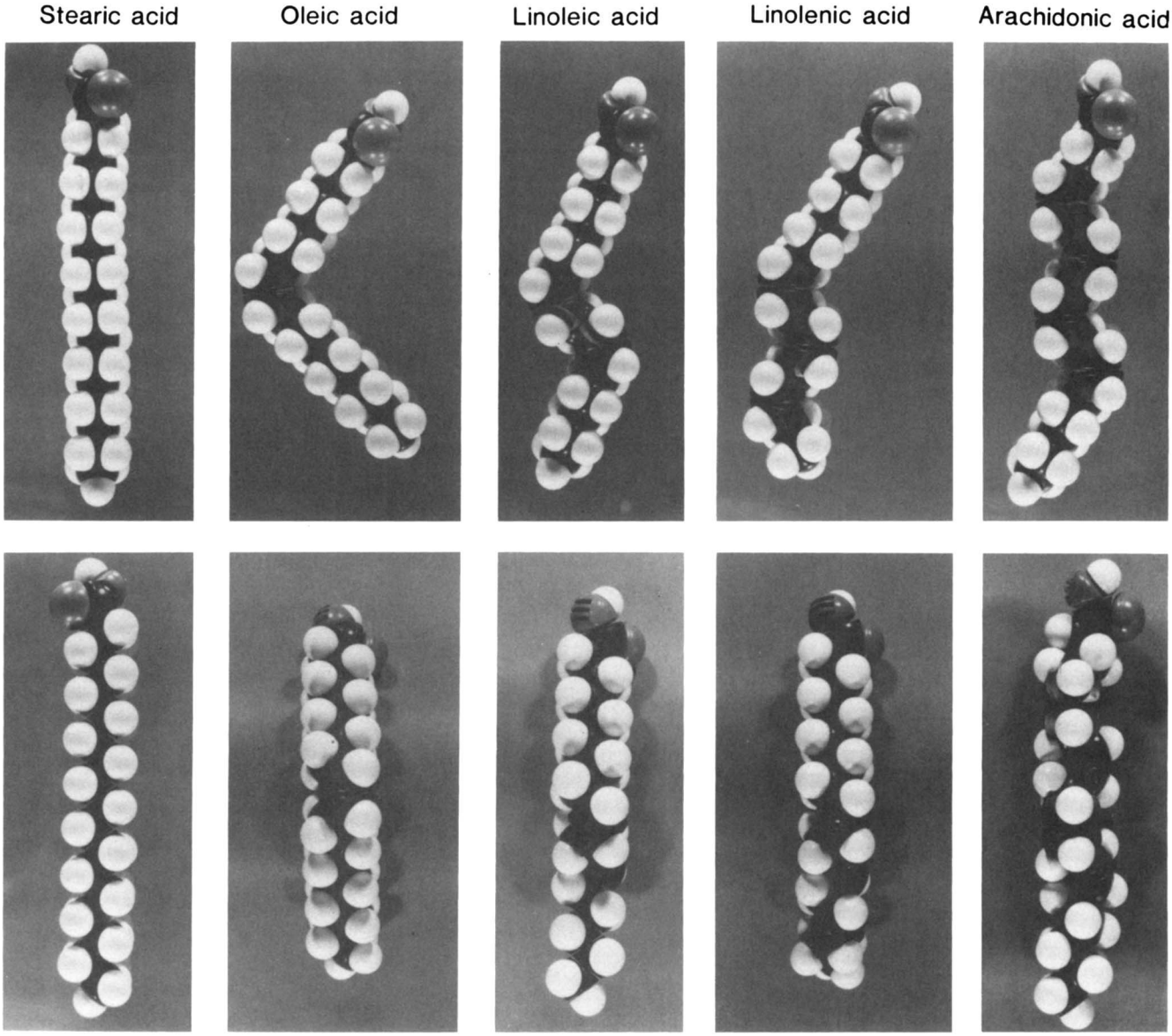

Fig. 1. Space filling models of five fatty acids. The conformation of five fatty acids of increasing unsaturation are shown from two different perspectives, one orthogonal to the other. The unsaturation increases in order from left to right as follows: $C_{18:0}$, octadecanoic acid (stearic acid); $C_{18:1}$, 9-octadecanoic acid (oleic acid); $C_{18:2}$, 9,12-octadecienoic acid (linoleic acid); $C_{18:3}$, 9,12,15-octadecatrienoic acid (linolenic acid); $C_{20:4}$, 5,8,11,14-eicosatretraenoic acid (arachidonic acid). The saturated fatty acid (stearic acid) is linear (1). Bottom view shows the zig-zag conformation with the angle between carbons being about 110°. The upper view (turned 90°) shows that all carbons are in line in regard to this plane. The top view, oleic acid (2), has the 2 ends of the chain at an angle of ~120° about the *cis* double bond. However, at 90° to this (bottom), the chain appears linear. Of course, the double bond projects up from the plane of the page. Linoleic acid (3) has a skew about the carbons on each side of the C=C, which allows the two double bonds to be *trans* to each other (top view). This, in turn, allows the chains at the two ends of the molecule to have the same direction. In the orthogonal projection (bottom), the molecule appears linear but the first double bond projects up from the page and the second down. The conformation of linolenic and arachidonic acid are similar to linoleic acid, because the carbons on each side of the C=C are also skewed (3). Note that these crystalline conformations are only one of many possible polymorphic forms, but at present the structures of other polymorphic forms of unsaturated fatty acids have not been elucidated.

Crystalline states characterized by specific chain-chain interaction

These crystalline states are characterized by a specific arrangement between the atoms in the hydrocarbon chain and are conveniently classed as subcells, in which a_s and b_s describe the two-dimensional lattice perpendicular to the chain axis, and c_s is usually two carbons long with a distance of approximately 2.54 Å. Chain packing

TABLE 1. Comparison of nomenclature for hydrocarbon chain packing in the solid and liquid state

Aliphatic Chain Interaction	Subcell Lattice[a]	Mean Vol/$-CH_2-$	Mean Surface Area/$-CH_2-$	Motion	Common Names[b]
Specific	Orthorhombic perpendicular Orthorhombic parallel Monoclinic parallel Triclinic parallel Hybrid cells	~24 Å^3	~18.8 Å^2	Very restricted	Alkanes = orthorhombic perpendicular, triclinic, monoclinic, β, γ Acids and amides = A, B, and C forms Mono-, di-, and triacylglycerols = β and β' forms
Nonspecific	Hexagonal or near hexagonal	~25.5 Å^3	~20 Å^2	Restricted	Alkanes = rotator phase, α Acids, alcohols, glycerides = α phase Phospholipids = gel phase, ordered phase, hexagonal phase, L_β [Tardieu et al. (46)] Soaps = gel phase Smectic B of some liquid crystals [Falgueirettes and Delord (47)]
Liquid	No lattice, but domains of roughly ∥ chains	29 to 30 Å^3	~23 Å^2	Fluid	Alkanes, acids, alcohols, di- and triacylglycerols = melt, neat liquid, isotropic liquid phase Monoacylglycerols and phospholipids = liquid crystal phase (lamellar or L_α, cubic, hexagonal I and hexagonal II, etc.) [Tardieu et al. (46)] Soap = neat, viscous isotropic or middle phase; liquid phases Cholesteryl esters = liquid crystal, fluid crystal or mesophase (smectic A and C, nematic, cholesteric), ordered (as opposed to isotropic liquid)

[a] For a good review of simple and hybrid subcells, see Abrahamsson et al. (6).
[b] Nomenclature for different states of lipids is complicated and not consistent between lipid classes. This table should be helpful in orientation since many aliphatic molecules undergo transitions from various crystalline states to more liquid-like states. For further discussion of specific states see Abrahamsson et al. (6), Tardieu et al. (28, 46), and Chapman (39).

in a two-dimensional rectangular lattice includes the following subcells: orthorhombic perpendicular (O⊥, O′⊥) and orthorhombic parallel (O∥, O′∥). Oblique two-dimensional lattices include the triclinic parallel (Tc∥) and the monoclinic parallel (M∥) subcells.

Varieties of hybrid subcells occur in more complex single chain lipids, and these have been summarized clearly by Abrahamsson et al. (6). In complex lipids, especially those with two or three chains in the same molecule, packing is restricted by the attachment of the chains to a common part of the molecule (e.g., glycerol, sphingosine). In some cases, the restriction leads to a super-lattice structure, consisting of several chains packed in a hybrid lattice. Chain packing in these super-lattices, however, retains characteristics similar to the simpler tightly packed chain lattices. **Fig. 2** depicts two typical arrangements of closely packed hydrocarbon chains, a triclinic subcell (Fig. 2a) and an orthorhombic subcell (Fig. 2b). These and other closely packed arrangements of hydrocarbon chains demonstrate similar characteristics. The mean volumes of the $-CH_2-$ groups are comparable between the different subcells (~24 Å^3 per $-CH_2-$ group) as are the surface areas perpendicular to the hydrocarbon chain axes (~18.8 Å^2 per chain). This similarity in volume and area is maintained to a great extent in the tightly packed crystalline forms of the most simple and complex aliphatic molecules, although twists and bends and regional differences in packing along the chain cause some minor variations (5).

Crystalline states in which specific chain-chain interaction is lost—nonspecific chain-chain packing

In this class of crystalline chain packing, which Müller (7) called the "rotator phase" of hydrocarbons, the aliphatic chains lose some of their specific interactions, and individual carbon atoms are able to rotate a few degrees within the lattice. Because of this partial rotation, the two-dimensional lattice and three-dimensional subcell pack hexagonally or nearly hexagonally (Fig. 2c). In systems at equilibrium, this packing occurs at higher temperatures than the more tightly packed phases described in the previous section, and both the volume per $-CH_2-$ group (approximately 25.5 Å^3) and the area per hydrocarbon chain (approximately 20.0 Å^2) are greater. As indicated, the hydrocarbon chains have more mobility, although they are still extremely restricted

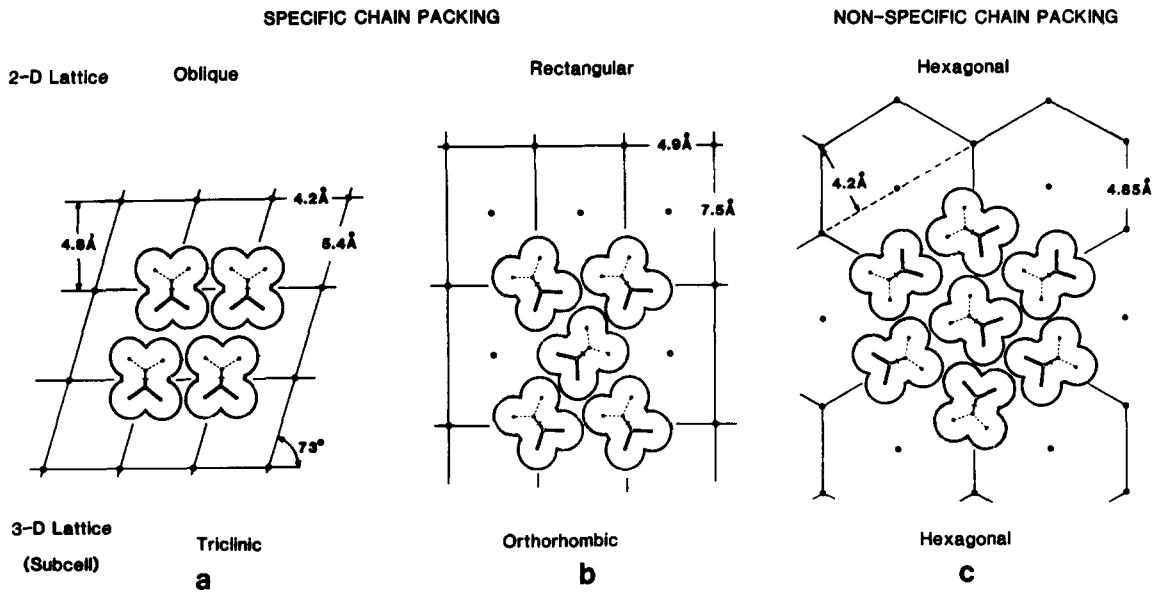

Fig. 2. Hydrocarbon chain packing in the solid state. 2a. Typical triclinic subcell. The two-dimensional lattice is oblique. This represents a tightly packed chain in which there is specific chain-chain interaction. The mean volume per $-CH_2-$ group is about 24 Å^3, and the area perpendicular to the chain is about 18.8 Å^2 per $-CH_2-$ group. 2b. Orthorhombic perpendicular subcell. Here, the two-dimensional lattice is rectangular, but this also represents a tightly packed lattice with specific chain-chain interaction. The volume and area per $-CH_2-$ group are similar to the triclinic subcell. 2c. Hexagonal subcell. The two-dimensional lattice is hexagonal, hence its name, and gives rise to a 4.1 to 4.2 Å reflection between the plane of the chains. Chain packing is loose, and specific chain-chain interaction is lost due to the ability of the carbon atoms to rotate several degrees and form gauche conformers. Assuming that the C–C bond distance along the chain is 1.27 Å, and the center-to-center distance of the chain is 4.85 Å, the volume of the $-CH_2-$ group is 25.9 Å^3 and the area perpendicular to the chain is 20.4 Å^2.

in comparison with the liquid state. There is an increase in gauche conformers in this phase and most are located towards the $-CH_3$ end of the chains (8).

Chain packing in the liquid state

The transition from the crystalline to the liquid state is accompanied by an absorption of heat, a sharp increase in molecular volume, and a loss of long-range order between the molecules. The liquid state of lipids shares the general properties of the liquid state of matter, including fluidity, cohesiveness, relative noncompressibility, and rapid molecular motion. The relatively small volume increase (in the order of 10 to 20 percent) occurring during the melting of the crystalline chains to liquid indicates that some short-range order must exist between the aliphatic chains in the liquid state. In fact, in the neat state of many liquids, X-ray scattering experiments show that domains of layered structures, roughly equivalent to the lengths of the molecules, are present in liquids. For instance, alkanes and halogen-substituted hydrocarbons have been shown by Brady's group to align in domains several hundred angstroms in size, with minimum dislocations in the liquid state (9–13). Furthermore, this group has shown quite clearly that when molecules of different configuration (short and long chain alkanes, or aromatic and aliphatic alkanes) are mixed in the liquid state, the domains of aliphatic molecules remain even at quite low concentrations, indicating a nonideality of solutions of hydrocarbons. That is, hydrocarbons of markedly different chain lengths or of different conformations tend to segregate into domains in the liquid state. Domains in the neat state have also been noted in triglycerides (14), soaps (15), and cholesteryl esters (16).

Transitions between tightly packed crystals, the rotator phase, and the liquid state

First-order phase transitions are associated with an excess specific heat and sharp increase in volume at the transition. The subtle differences between states and their transitions are amply illustrated in **Fig. 3** (17) by three alkanes differing in length by only two carbons ($C_{21}H_{44}$, $C_{22}H_{46}$, $C_{23}H_{58}$). The differential scanning calorimetry (DSC) traces are given at the top, the unit cell dimensions are plotted in the middle, and the structures are illustrated at the bottom of the figure. The unit cell data are taken from Doucet, Denicolo, and Craievich (18, 19) and from Denicolo, Doucet, and Craievich (20). The DSC traces were performed in our laboratory.

$C_{21}H_{44}$ has two first-order transitions (Fig. 3, left side). Below 32°C, the structure is orthorhombic perpendicular ($O\perp$). Above the lower transition at 32°C, the a and b cell dimensions indicate a two-dimensional rectangular lattice. The lattice dimensions change with increasing temperature towards, but not reaching, true hexagonal packing ($a/b = \sqrt{3}$) before melting occurs at 40.5°C.

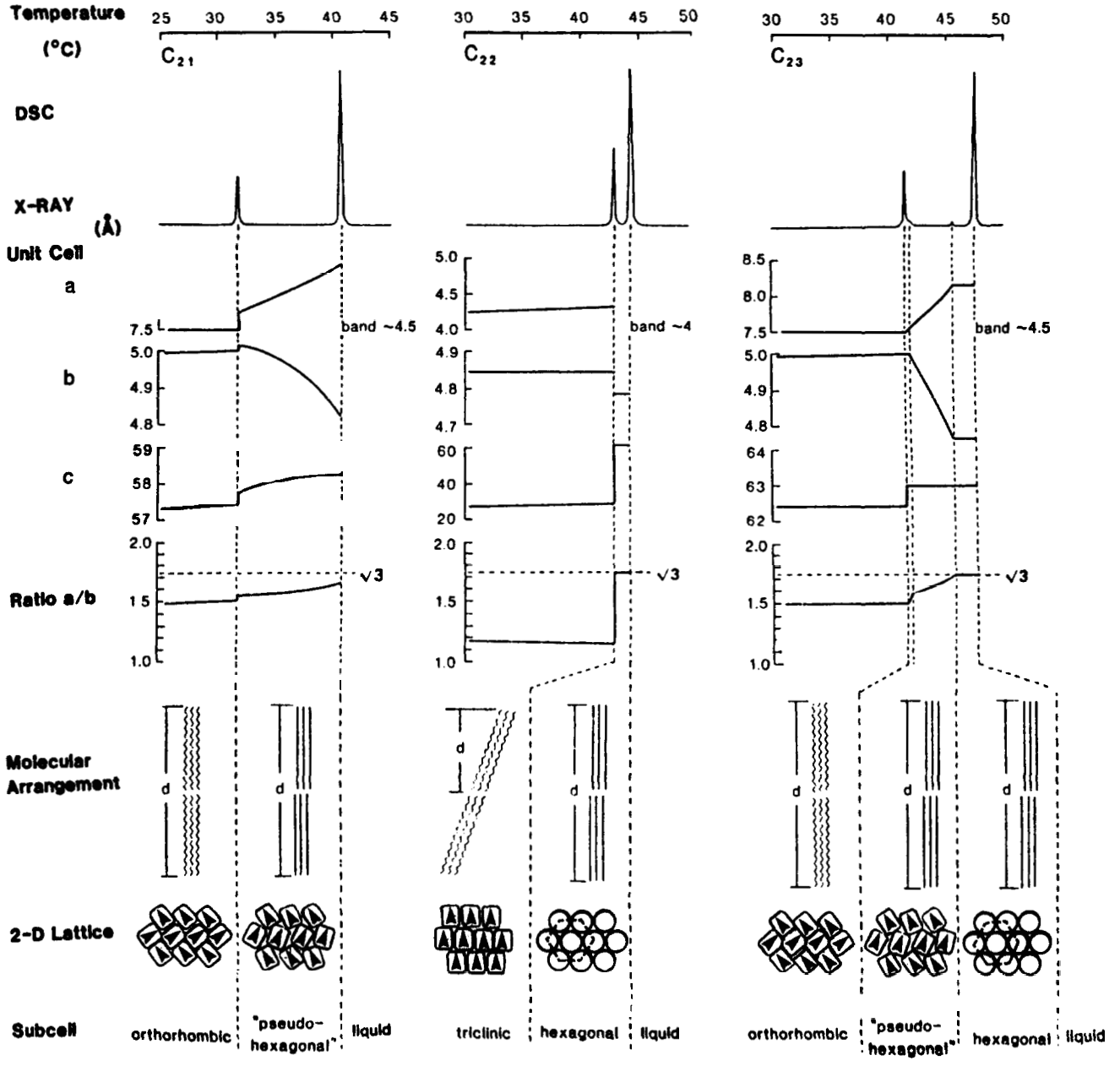

Fig. 3. Comparison of three n-alkanes in which stable rotator phases are present. a. n-heneicosane (C_{21}); note that only the "pseudohexagonal" rotator phase is present; X-ray data of Doucet et al. (18). b. n-docosane (C_{22}); only the hexagonal rotator phase is present; X-ray data of Denicolo et al. (20). c. n-tricosane (C_{23}); both rotator phases are present; X-ray data of Doucet et al. (19) and Ungar (45). The unit cell parameters determined by X-ray diffraction indicate that two rotator phases can exist, one with pseudohexagonal and the other with true hexagonal chain packing. The transition between these two forms is of very low energy (see DSC of C_{23}). Representations of the lamellar characteristics in each phase, where "d" is the crystallographic repeat distance, and representations of the subcell packing at the lamellar plane are given below. The odd chain alkanes (a and c) have an orthorhombic perpendicular (O⊥) subcell in the crystal phase, while the even chain alkane (b) subcell is triclinic (Tc). The repeat distance of the odd alkane crystalline state, as well as the repeat distance of all rotator phases, is equivalent to two molecular lengths. The repeat distance for even carbon alkanes in the crystal state is only equivalent to one molecular length.

$C_{23}H_{48}$ shows three potential first-order transitions (Fig. 3, right side). The two large transitions at about 41°C and 49°C are analogous to those for $C_{21}H_{44}$, i.e., from orthorhombic perpendicular to pseudohexagonal and melting. The very small transition at ~45°C of about 30 cal/mole coincides with the transition from pseudo to true hexagonal packing. The X-ray studies of Doucet et al. (18, 19) illustrate two basic lattices for the rotator phase, a pseudohexagonal or a two-dimensional disordered rectangular packing of the chains below 45°C and a true hexagonal packing between 45° and 49°C. In contrast to $C_{21}H_{44}$, the lattice parameters of $C_{23}H_{48}$ change as temperature is increased in the rotator phase such that *a* increases and *b* decreases, and the

ratio of a/b becomes $\sqrt{3}$ at 45°C, which indicates that true hexagonal symmetry is reached. Thus, the orthorhombic perpendicular subcell lattice just above 41°C is distorted, so that the two-dimensional chain packing lattice is a slightly disordered rectangular lattice. As the temperature is increased above 41°C, the two-dimensional rectangular lattice gradually approaches hexagonal symmetry. At the 45°C transition, the chain packing is truly hexagonal. In fact, odd hydrocarbons between C_{23} and C_{27} all have evidence of a small phase transition, occurring in the rotator phase, corresponding to pseudo- to true hexagonal packing.

Long-chain even hydrocarbons ($>C_{20}$) have a triclinic chain packing which has a transition to a true hexagonal packing ($a/b = \sqrt{3}$) (19). Furthermore, the c-axis, as reflected by the long spacings, more than doubles at the transition from triclinic to rotator phase, indicating that each layer of molecules is displaced relative to the one above it. Subtle changes in the rotator phase also appear to show up in the infrared spectra; Snyder et al. (8) noted a discontinuous, sudden increase in the number of gauche conformers in the C_{23}–C_{29} odd alkanes at about the correct temperatures. The changes in heat content are minor (19). I have measured only 20–30 cal/mol in C_{23} and C_{25}.

Thus, the structure of the rotator phase depends not only on chain length but also on the crystalline structure below the rotator phase. $C_{21}H_{44}$ and shorter odd-numbered hydrocarbons have a relatively low polymorphic transition from O⊥ to a pseudohexagonal rotator phase, which in turn melts before reaching true hexagonal symmetry. $C_{23}H_{48}$ and longer odd alkanes have a higher melting point which allows the pseudohexagonal phase to reach true hexagonal symmetry before melting. In even hydrocarbons, such as C_{22}, the relatively higher polymorphic transition from triclinic to rotator phase eclipses the pseudo-hexagonal temperature domain, and only the hexagonal chain packing is seen. Such subtle changes in chain packing have also been noted in other lipids, particularly in phospholipids (21), and are important to chain packing in the more complex molecules, where the chains are forced to pack together because of their linkage through glycerol (i.e., triglycerides and phospholipids).

Olefinic chains

Only a few single crystalline studies have been performed on lipids with olefinic or unsaturated double bonds. The structure of the low melting form of oleic acid, whose conformation is shown in Fig. 1, was determined by Abrahamsson and Ryderstedt-Nahringbauer in 1962 (2). The hydrocarbon chains pack in an orthorhombic parallel configuration. The crystalline structure of linoleic and some other polyunsaturated fatty acids have been studied by Ernst, Sheldrick, and Fuhrhop (3), and apparently the packing in this state is similar to oleic acid (see Fig. 1). The crystalline structure of cholesteryl oleate has been carried out by Craven and Guerina (22), but the chains in this structure do not pack in any known subcell.

Interaction between different kinds of chains

A direct way of studying the interaction of chains is by studying the phase equilibrium of binary mixtures of molecules of different chain lengths, for instance, short and long chain alkanes or short and long chain fatty acids. If the molecules form a continuous solid solution in the crystalline state, then one can assume that they mix in the crystalline state. On the other hand, if the different molecules segregate into separate phases, excluding the opposite molecule from the crystalline phase, then mixing does not occur in the crystalline state. As one might predict, chains that are very similar in chain length, i.e., differing by less than four carbon atoms, tend to mix in continuous solid solutions. When the chain length is greater than four carbons, however, the solid solutions become partial and regions of nonmixing occur with separation of two solid solutions, one rich in one component, the other rich in the other component. When the chains are not too different, they can dissolve a small amount of the opposing molecule within the crystalline structure. In many cases the domain of nonspecific crystalline structures, that is, hexagonal chain packing, is markedly increased by chains with slightly different chain lengths. However, when chains differ in length by six or more carbons, they tend to be immiscible in the solid state and to separate into simple monotectic or eutectic systems. Phase diagrams of binary mixtures of molecules, whose chains differ by greater than six carbons, have been studied in alkanes, n-alcohols, fatty acids, triacylglycerols, and phospholipids, and in every case, phase separations occur in the crystalline state, indicating nonmiscibility of long and short chains in the crystalline state (see, for example, 17,23–26).

Interaction of saturated and unsaturated molecules in the solid state

Perhaps the most revealing phase diagram is the alkane-alkene system recently studied by us (17). The two molecules studied were octadecane and 9-octadecene. The phase diagram shown in **Fig. 4** is a monotectic system in which there is virtually no miscibility of octadecane and octadecene in the solid state. In these systems there is no polar part to align or interact to form potential polar-polar interactions. Therefore, one can conclude from this diagram that, in the solid state, saturated and mono-unsaturated hydrocarbons of approximately the same chain length do not mix. Note that nonspecific hexagonal chain packing does not occur in this system. Thus, saturated and unsaturated chains do not like to mix in the solid state. The same nonmixing also occurs when there is hydrogen bonding. For in-

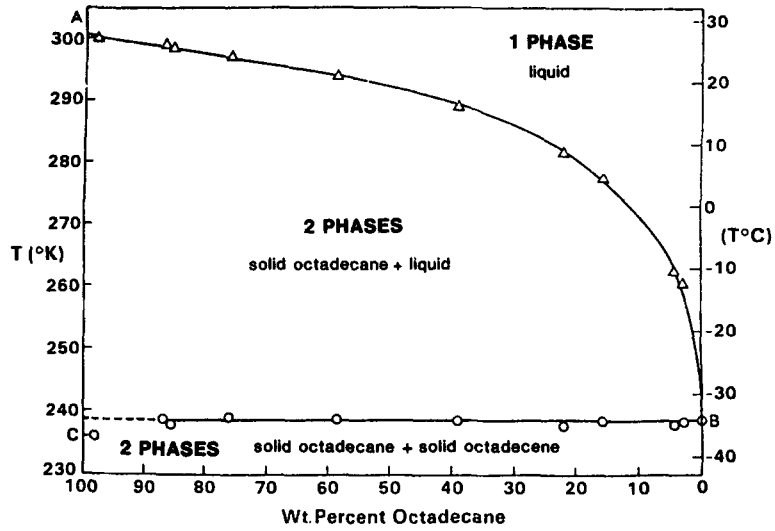

Fig. 4. Octadecane-octadecene phase diagram. The system is monotectic; in the crystal state, no interactions take place. Crystals of octadecane separate from crystals of octadecene. (D. M. Small, J. Steiner, and J. Hamilton, unpublished observations).

stance, in the phase diagram of oleic acid and palmitic acid published by Bailey (25), there is no apparent mixing in the solid state (24, 26). Even in more complex systems, such as triolein and tristearin, nonmixing occurs in the solid state. Thus, as a general rule, aliphatic and olefinic chains do not pack together in the solid state. While no definitive data exist on the liquid state, it is probable that there are domains of saturated chains separated from unsaturated chains in the liquid state, and that these domains would serve as nucleating centers for crystalline solids of the highest melting of the two components.

Packing of two different chains connected covalently

Most biological molecules, complex lipids, such as phospholipids, di- and triacylglycerols, wax esters, and so forth, contain two or even three different hydrocarbon chains. Often one or more chain is saturated and the other is mono- or polyunsaturated. However, these chains are connected through covalent bonds, either as an ester bond or through glycerol or sphingosine. Taking into account that widely different chains do not like to pack together, how do they pack together when forced to through covalent connections? Two possibilities can occur in molecules that have two different chains, such as waxes, diglycerides, and phospholipids. One is that the chains can segregate, or point in opposite directions from the point where they are united, forming separate layers in which the different chains are sequestered. This circumstance is illustrated in **Fig. 5a** with a wax ester having one saturated and one unsaturated chain. The saturated and unsaturated chains crystallize in different layers and are thus segregated. This type of structure might also occur with complex chain diacylglycerols. On the other hand, in aqueous systems of phospholipids or sphingolipids, the chains are forced to lie parallel (i.e., to turn back on themselves), because water orients the polar group and prevents one of the chains from pointing in the opposite direction (i.e., into the water space). This circumstance forces the different chains to lie roughly side by side, and therefore chains of different length, and straight and kinky (unsaturated) chains, are forced to interact.

When the aliphatic chains differ only in chain length, the packing can be adjusted by tilts or alterations in the structure of the hydrocarbon layer. For instance, when both chains are of similar length, hexagonal chain packed para-crystalline phases occur, in which the chains are tilted (27, 28) (Fig. 5b). In cases in which the polar group is large, the bilayers are rippled, accommodating for the presence of the polar group (28–30). Crystallization into more specific chain-chain packing occurs at lower temperatures with the exclusion of water and, presumably, specific hybrid lattices (31–33) similar to those found for the crystalline tetrahydrate described by Pearson and Pascher (34) are formed. However, equilibration is slow and only occurs at one appropriate temperature range. When the chain on the two position is extremely short, like acetate or proprionate, then interdigitation of the layers probably occurs as in lysolecithin (35), producing a single interdigitated layer of lipid in the gel phase (Fig. 5c). However, when the chain on the two position is about half the length of the chain on the one position, for example, with 1-stearoyl,2-caproyl lecithin, then the gel phase that is formed is quite different (Fig. 5d); the stearoyl fatty acid crosses the entire bilayer, and the two C_{10} chains meet end to

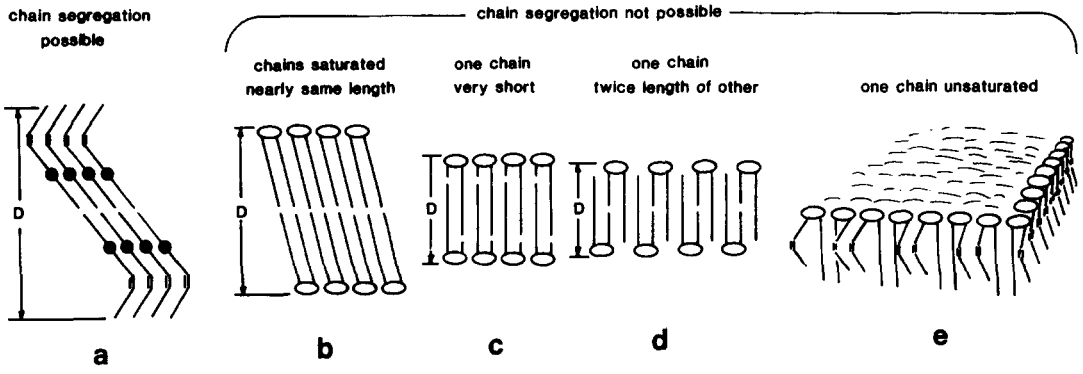

Fig. 5. Possible structures of lipids containing two different aliphatic chains. a) A wax ester or a diacyl glycerol in the neat state may crystallize with two chains segregated into separate bilayers. The subcells of each separate bilayer would be different. Figures b, c, d, and e indicate the possible chain packings when chain segregation is not possible. These systems occur in hydrated systems in which the acyl chain is prevented from entering the aqueous phase by a strong interaction of the polar group with water. Such systems are formed by phospholipids or glycolipids. b) If the chains are nearly the same length they can pack in the standard L'_β, P'_β, or even crystallize into even more specific chain packing, such as that found in hybrid cells. c) When one chain is long and the other very short, the chains may interdigitate, forming a system analogous to lysolecithin in which the bilayer thickness is slightly greater than one chain length. In this structure the packing is hexagonal. d) The 1-per-3 packing described by McIntosh et al. (36) occurs when one of the acyl chains is approximately twice the length of the other. The long chain spans the bilayer and the two shorter chains meet at the center. This results in each polar group covering the area of three chains, thus the 1-per-3 structure. In the 1 and 3 structure, the chain packing is hexagonal. e) Possible packing in the case of one saturated and one unsaturated chain of approximately the same length (e.g., in palmitoyl oleyl lecithin). The possible packing shows both the saturated and the unsaturated chains lining up in pairs within the plane of the bilayer making maximum chain segregation possible with this system. Random alignment of all chains frozen in a loose hexagonal packing might also be possible, but would be less stable.

end, so that the number of chains per head group is three instead of two (36). This 1-per-3 arrangement permits the molecules to align perpendicularly to the plane of the bilayer. The chain packing is hexagonal (36).

When the chain in the two position is unsaturated, for instance, in the case of stearoyl-oleoyl phosphatidylcholine, or a stearoyl-linoleoyl phosphatidylcholine, then the chains must lie side by side and interact. There is no doubt that a gel phase with a strong 4.2 short spacing is present in egg lecithin and in palmitoyl-oleoyl lecithin. However, more precise crystalline structures, such as those with specific chain-chain interaction, have not been found, although they have not been looked for in detail. The enthalpy (or the entropy) of the transition from gel to liquid crystal for several unsaturated lecithins, in which the one position is stearate and the two position is oleate, linoleate, linolenate, or arachidonate, has been studied by Coolbear, Berde, and Keough (37). Some acyl migration and the presence of small amounts of fatty acid made precise determination of the enthalpies impossible but, in general, the enthalpies were about half that found for distearoyl lecithin, i.e., about 3–6 kcal per mole versus 10 kcal per mole. Although no X-ray studies were done, on the basis of diffraction studies of egg lecithin, they are probably gels with hexagonal chain packing. Possibly, within the bilayers, rows of two stearoyls, then two oleoyls, then two stearoyls exist, permitting some linear segregation to occur within the bilayer itself (Fig. 5e). It seems unlikely that completely random chain distribution is present in the gel phase. The fact that the enthalpies of the transitions are rather similar for molecules with as few as one double bond to those with four double bonds, probably indicates that the conformation of the polyunsaturated fatty acid retains its more or less linear conformation (shown in Fig. 1) in the bilayer, and does not bend back on itself as has been suggested. Certainly, a nonlinear, nearly cyclic structure would be less compatible with the bilayer structure.

Presence of more than two chains on a single molecule

Triacylglycerols form a prime example of the possibility of having three separate chains attached covalently to the same glycerol molecule. When two of the chains in the triacylglycerol are similar, as shown in **Fig. 6,** then chain segregation can and does occur readily, with the odd chain forming an interdigitated monolayer and the two other chains forming a bilayer (24, 37, 38). Conformation around the glycerol must change if the odd chain is on the two as compared with the one or three position (38). However, chain segregation is clearly possible. The X-ray spacings of such molecules indicate that a trilayer is present (38, 39).

Triacylglycerols with three very different chains are quite prevalent in biological systems (40). They do crystallize and often with evidence of specific chain-chain packing (e.g., β or β' short spacings) (25, 41, 42), but little is known of their definitive crystalline structure. As mentioned above, triacylglycerols of the type $C_nC_xC_n$, and probably $C_xC_nC_n$, where C_x is very different from

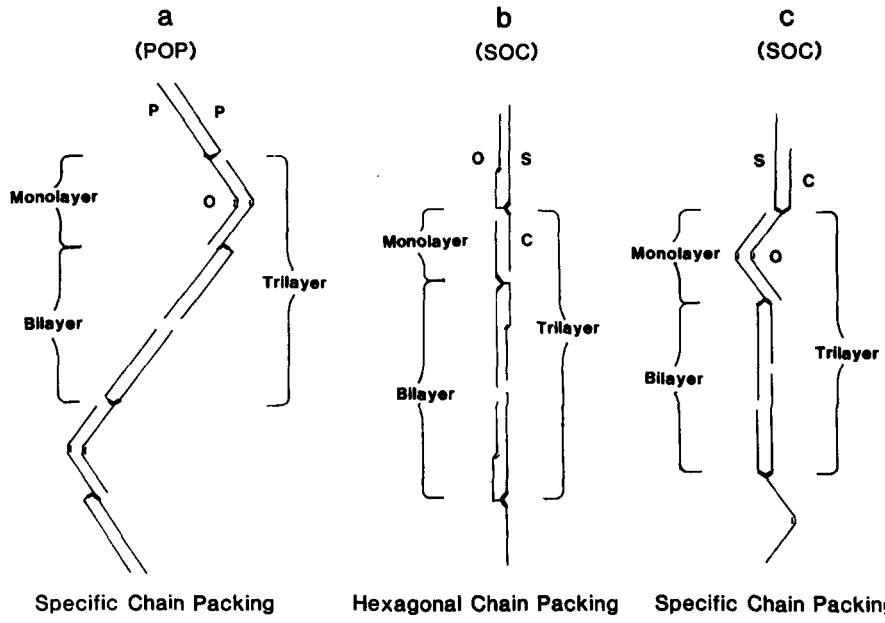

Fig. 6. Some possible structures for complex triacylglycerols. Primitive trilayered crystal structures for (a) 1,3-dipalmitoyl-2-oleyl-1-glycerol (POP); and (b, c) for two possible configurations of *sn*-1-stearoyl-*sn*-2-oleyl-*sn*-3-caproyl-glycerol (SOC). (a) POP can form a primitive trilayered structure because the unsaturated chains segregate in a monolayer and the saturated chain in a bilayer (the true unit cell would actually be six layers). The subcells of the mono- and bilayers are probably different. For instance, the oleic acid layer might be orthorhombic parallel (O‖), while the palmitates might be triclinic (Tc‖). The wide-angle X-ray diffractions would be complicated because spacings would be present for both layers, and the intensity of the spacings of the palmitate layer would be about twice that for the oleic acid layer. (b) An α-form of SOC with nonspecific hexagonally packed chains. The trilayer consists of a monolayer containing the segregated short chains and a bilayer containing the longer acyl chains in nonspecific hexagonal packing. (c) A probably more stable form of SOC with specific chain packing. The monolayer contains the segregated unsaturated acyl chain in an O‖ subcell and a bilayer interdigitated saturated chain in an O⊥ or T‖ subcell. In this case, two trilayers are required to form a unit cell. The second trilayer is a mirror image of the one shown.

C_n, tend to form trilayers, segregating the C_n acyl groups in a bilayer and the C_x acyl groups in an interdigitated monolayer (Fig. 6a). Consider a molecule such as *sn*-1-stearoyl-2-oleyl-3-caproyl-triacylglycerol (SOC) (Fig. 6b, c). All three acyl groups are unique. Further, if mixed as individual chains, they would tend to crystallize as separate structures and not co-crystallize into solid solutions. However, since all three chains are covalently linked through glycerol, it is not clear just how SOC will crystallize. Crystallization might produce a trilayered structure in the α-form (nonspecific hexagonally packed chains), for instance, with the two C_{18} fatty acids co-crystallized and segregated from an interdigitated monolayer of the short chains (Fig. 6b). A more stable crystalline form, with all three chains having specific chain-chain interaction, might also form a trilayered structure in which an interlaced bilayer of C_6 and C_{18} with O⊥ or Tc subcells and a segregated interdigitated monolayer of the oleyl chains having O‖ subcell packing would coexist (Fig. 6b). Finally, it is conceivable that these molecules do not form a standard layered system at all, but pack in such a way that all three chains segregate and pack in a quite different lattice.

Finally, some triglycerides contain two different unsaturated chains, for example, palmitoyl-linoleoyl-arachidonyl glycerol, and it is not clear how such systems would pack in a specific lattice, although it is possible that a specific interdigitated layer of the saturated fatty acids could be present and a bilayer of nonspecific chain packing accommodating the unsaturated fatty acids. This would give a trilayered structure with evidence of both specific chain-chain packing in one layer and nonspecific chain packing in the other layers.

SUMMARY

The aliphatic chains of many biologically important lipids are heterogeneous and often related to the functions of the molecules. Certain phospholipids containing arachidonic acid may serve as precursors for prostaglandins, certain diglycerides may serve as second messengers for certain membrane-triggered reactions (43), and other phospholipids containing a very short chain in the two position may serve as vasoactive hormones (44). The packing of such molecules is of interest. The evidence is quite clear from both the conformation of saturated

and unsaturated molecules and from mixing experiments in the solid state that long and short chains don't mix well, nor do unsaturated and saturated chains, even if they are of the same chain length. There is even some evidence to indicate that some degree of chain segregation occurs even in the liquid state. However, different chains are often associated through covalent bonds, e.g., in wax esters, diacylglycerols, triacylglycerols, and phospholipids. A variety of possibilities for chain segregation are present in the neat phases of wax esters, ceramides, diacylglycerols, and triacylglycerols. However, in the unique case of membrane lipids like phospholipids or sphingolipids, the two chains are forced to lie side by side by virtue of the interaction of the polar group with water, and thus interactions between different chains must occur. Most of the evidence suggests that, when a solid phase results in these systems, the nonspecific chain packing mode (hexagonal chain packing) is preferred. In fact, for all of the phospholipids studied thus far, clearcut evidence of specific chain-chain interaction in molecules having both unsaturated and saturated chains has never been observed. However, for mixed chain triacylglycerols, evidence of specific chain-chain interactions (β' and even β) has been found and some suggestions have been given as to how this might occur through chain segregation mechanisms in the neat state. The literature suggests that further work needs to be done on the interaction of different chains that are covalently linked to the same molecule. Such studies will lead to a better understanding of the structure of lipid bilayers, membranes, lipoproteins, and lipid deposits.

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