Chapter 9 - Lipids and Biological Membranes

Introduction:

Lipids constitute the fourth category of biomolecules and, unlike proteins, nucleic acids and carbohydrates, they are not polymers. They are defined experimentally as those biomolecules that are extracted with organic solvents and so, not surprisingly, exhibit a lot of variety. They have three main biological functions:

1. Important components of biological membranes (phospholipids, glycolipids)
2. Important energy stores (triglycerides)
3. They are involved in intra- and intercellular signalling events.

Fatty Acids

Fatty acids are long chain carboxylic acids, \( \text{CH}_3(\text{CH}_2)_{n-2}\text{COOH} \), where \( n \) is usually even and typically is between 14 and 24. Shown below are two fatty acids, stearic acid (or stearate) and linoleic acid (linoleate). Stearate is saturated; linoleate is polyunsaturated and is an omega six fatty acid.

![Fatty Acid Structures]

Table 9-1 lists several common biological fatty acids. You should know how to draw palmitate, stearate (unsaturated), oleate (monounsaturated) and linolate and linolenate (polyunsaturated). Note that unsaturated fatty acids almost always have cis double bonds. Trans fatty acids are a byproduct of partially hydrogenated vegetable oils and have been known to be a risk factor for heart disease. Note from Table 1 that melting points of fatty acids increase with chain length and decrease with increasing unsaturation. You will find that melting points of lipids derived in part from fatty acids (triacylglycerols and phospholipids, for example) also follow this trend.

Recall that fatty acids are amphipathic molecules and form micelles in solution.

Triacylglycerols (Triglycerides)

Fatty acids are highly reduced molecules and as such are important sources of energy.
Due to their tendency to form micelles fatty acids are stored as triglycerides, also known as fats, which are fatty acid triesters of glycerol. Tristearin is shown below:

![Tristearin structure](image)

Triglycerides are highly insoluble and are stored as cellular droplets in adipocytes, or fat cells. Not only are triglycerides more highly reduced than the other main energy stores, carbohydrates, they are also less soluble, hence have less water of hydration associated with them. For this reason, triglycerides provide about six times the metabolic energy of an equal weight of hydrated glycogen (which, you may recall, is the form in which we store carbohydrate (animal starch)).

Melting points of fats depend on the chain length and degree of unsaturation of their fatty acids. Tristearin, the major lipid of animal fat, is a solid at room temperature, which you will notice when you brown ground beef, drain the fat, and let it cool down. Vegetable oil, which contains unsaturated fatty acids is a liquid at room temperature.

**Glycerophospholipids**

These are major components of biological membranes. They consist of glycerol + 2 fatty acids + phosphate + R group (figure 9-3 b),

![Glycerophospholipid structure](image)

where the R group is an alcohol, such as ethanolamine, choline, serine, etc. Serine and inositol are shown below. See Table 9-2 for a complete list.
Figure 9-4 shows the structure of a phosphatidyl choline (PC).

Other glycerophospholipids are abbreviated PS, PI, PE, etc.

Glycerophospholipids, in addition to being important components of biological membranes, as we’ll see in Chapter 10, are also important biological surfactants. For example, cellular droplets of triglycerides in adipocytes are surrounded by a monolayer of phospholipid. Also, PC’s, in particular DPPC are important lung surfactants, where they form monolayers on the surfaces of cells that form the small air spaces of the lungs (alveoli), thereby decreasing the surface tension, which facilitates breathing (see box 9-1).

Phospholipases are enzymes that cleave phospholipids. Various venoms contain
phospholipases. Their action is summarized in Figure 9-5:

**Note:** The hydrolysis products of membrane lipids can lead to the production of signaling molecules, such as secondary messengers. For example, the production of lyso-PA in blood platelets and injured cells stimulates cell growth. Also, the action of a phospholipase C leads to the production of 1,2-diacylglycerol, an intracellular messenger that activates a protein kinase.

A plasmalogen is a phospholipid with the fatty acid at C-1 attached via an ether, rather than ester linkage, with unsaturation at the \(\sim\) position (ethanolamine, choline and serine are the most common head groups).

The vinyl group is easily oxidized, hence plasmalogens may react with oxygen free radicals, thus preventing free-radical damage to other cell constituents.
**Sphingolipids**

These phospholipids are based on sphingosine, rather than glycerol

- Ceramide = N - fatty acid derivative
- Sphingomyelin = (typically) ceramide + Phosphate + choline or ethanolamine
- Cerebroside = ceramide + single sugar
- Ganglioside = ceramide + oligosaccharide

A sphingomyelin is shown below. Sphingomyelins are important membrane components.

Cerebrosides and gangliosides are glycosphingolipids. Both cerebrosides and gangliosides lack phosphate groups; rather, they are both contain mono- (cerebrosides) or complex saccharides (gangliosides) bonded to a ceramide moiety via an O-glycosidic linkage. A ganglioside is shown in Figure 9-9.
Note: Gangliosides on the external surface of membranes act as specific receptors for certain pituitary hormones that regulate a number of important physiological functions. They are also receptors for certain bacterial toxins, such as cholera toxin. They also appear to function in cell-cell recognition, thus are likely involved in growth and differentiation, and also carcinogenesis. Several genetic disorders of ganglioside breakdown are known, such as Tay-Sachs disease.

**Steroids**

Cholesterol (Figure 9-10) is also a major component of animal plasma membranes. The cholesterol is an amphipathic molecule due to the presence of the hydrophilic OH group.
the metabolic precursor of steroid hormones:

Glucocorticoids, such as cortisol, and mineralocorticoids, such as aldosterone, are produced in the cortex (outer layer) of the adrenal gland. Glucocorticoids affect carbohydrate, protein and lipid metabolism. Also affect inflammatory reactions, stress response, etc. Mineralocorticoids regulate excretion of salt and water by the kidneys (Figure 9-11).

Androgens and estrogens affect sexual development and function (Figure 9-11) Both androgens and estrogens are produced by the testes and ovaries, although the testes produce primarily androgens and the ovaries primarily estrogens.

Note that hormones are cellular messengers that mediate the coordination of metabolic activity in complex organisms. Steroid hormones are water-insoluble, unlike water-soluble hormones such as the catecholamines (produced in the inner portion of the adrenal gland (medulla)), and thus bind to and are transported through the blood by proteins.

Vitamin D is also produced from cholesterol and is involved in Ca\(^{++}\) metabolism. It is produced from a cholesterol derivative shown below. UV radiation cleaves a bond in the steroid nucleus; subsequent spontaneous rearrangement of double bonds, followed by enzymatic hydroxylation (liver) produces the active form, which increases serum Ca\(^{++}\) concentrations by stimulating uptake of dietary Ca\(^{++}\) by promoting its absorption in the intestine, and also by stimulating its release from bone. Rickets is a nutritional disease caused by insufficient bone mineralization and causes stunted growth and deformed bones in children. In the early 20\(^{th}\)
In the 19th century it was shown that rickets could be prevented by including animal fats in the diet. Exposure to sunlight also prevents rickets.

Note that excessive intake of vitamin D, like other fat-soluble vitamins, can be dangerous because it is retained in body lipids. Excessive intake of vitamin D can lead to vitamin D intoxication, resulting in bone demineralization, which then becomes fragile, abnormal calcification of soft tissues, kidney stones and failure. High levels of skin pigmentation in populations near the equator may be a protection against vitamin D intoxication.
Other Lipids

Isoprenoids are built from isoprene.

\[
\begin{align*}
\text{CH}_3 \\
\text{H}_2\text{C} & \text{CH} \\
& \text{C} \text{CH}_2
\end{align*}
\]

They include coenzyme Q, a component of electron transport (see text, p. 245) and fat-soluble vitamins such as vitamin A (vision), derived mainly from beta-carotene, vitamin K (blood clotting) and vitamin E (anti-oxidant).

Eicosanoids

**Arachidonic Acid (20:4 del 5,8,11,14)**

(stored as C2 ester of PI and other PL)

- Aspirin
- Introduce Oxygen

- Prostacyclin
- Prostaglandin
- Thromboxane

Prostacyclins, prostaglandins and thromboxanes, known collectively as eicosanoids, are hormone-like substances whose cellular response are often mediated by c-AMP, produce a variety of effects: Inflammatory response, production of pain and fever, regulation of blood pressure, induction of blood clotting, control of reproductive functions such as the induction of labor, regulation of the sleep-wake cycle.

Lipid Bilayers

Several classes of the lipids we’ve discussed are amphipathic, such as fatty acids, phospholipids, and even cholesterol, whereas others, such as triglycerides, are very hydrophobic. Fatty acids aggregate to form micelles, as we’ve seen. The thermodynamic driving force for micelle formation is primarily the hydrophobic effect (also responsible for the globular nature of globular proteins), thus is largely entropic. Recall that \( G = H - T \) \( S \), and that \( G \) must be negative for spontaneous micelle formation. \( H \) for micelle formation is small (negligible
enthalpic contribution), whereas $S$ is fairly large and positive (entropically favorable). This is because water becomes much less structured if it is removed from intimate contact with hydrophobic groups (figure 9-13).

The hydrophobic effect is largely responsible for spontaneous assembly of amphipathic phospholipids into amphipathic, noncovalent strutures as well. Phospholipids, however, spontaneously assemble into bilayers because of the added steric effects of the additional aliphatic R group. Figure 9-14 demonstrates the steric crowding resulting from the additional aliphatic “tail.”

The crowding is relieved by formation of a bilayer. Bilayer sheets will spontaneously form water-filled enclosures. 9-15.
Phospholipid bilayers are noncovalent, dynamic structures in which individual lipids are mobile. Types of lipid movement are transverse diffusion (flip-flop), which is slow, and lateral diffusion, which is much faster. Lateral and transverse diffusion are illustrated in Figure 9-16.
The hydrophobic core of a phospholipid bilayer present in biological membranes is rather fluid, with a viscosity similar to that of light machine oil. The aliphatic, hydrophobic tails are thus somewhat mobile, with mobility decreasing toward the phosphate group. The fluidity of the core is controlled by acyl chain length and degree of unsaturation. Chain length decreases fluidity, unsaturation increases fluidity.

Phospholipids undergo a cooperative phase transition as temperature is changed, similar to ice \( \rightarrow \) water. Below the transition temperature the core of the membrane is in a solid-like, or gel state. Above the transition temperature, the lipid acyl groups are much more mobile. Since the phospholipids are still constrained to remain within their monolayer, the fluid state is often called a liquid crystal (Figure 9 - 18).

Bilayers form barriers to passage of hydrophilic solutes. They are selectively permeable, which allows different compositions to be established in cells with respect to their environment, and also in the organelles of eukaryotic cells.

Biological membranes contain proteins as well as lipids. Their functions include:

- Mediating transport processes. It is such proteins that allow membranes to be selectively permeable, allowing passage to some solutes, but not others.
- Receptors
- Energy transduction. Oxidative phosphorylation and photosynthesis are membrane-associated events which involve energy transduction. The mechanisms of the energy-storage process involve building up gradients, or concentration differences, of protons, hence the need for a membrane.

**Membrane Proteins**

Membrane proteins are classified as either integral (extracted with detergents), or peripheral (extracted by changing pH, ionic strength). As the names imply, integral proteins are embedded to varying extents in the bilayer, whereas peripheral proteins are superficially bound to the bilayer surface.

**Integral Membrane Proteins**

Integral membrane proteins are difficult to work with because they tend to aggregate and precipitate in solution when removed from their bilayer. Notice that the membrane-spanning
portion of glycophorin (Figure 9-20) consists exclusively of amino acid residues with nonpolar, hydrophobic side chains:

Notice also that this is one of three domains, the others being the exterior, C-terminal domain, as well as the interior, N-terminal domain. As would be expected based on their role in recognition processes, the carbohydrate residues (green diamonds) on this glycoprotein reside in the exterior domain. The interior domain of glycophorin is alpha helical because of the intramolecular H-bonding that characterizes alpha helices.

Figure 10-3 shows a hydropathy plot for glycophorin. A hydropathy is a quantitative measure of the hydrophobicity or hydrophilicity of a given amino acid residue, and is determined by partitioning the amino acid between water and a hydrophobic solvent such as chloroform. The relative amounts in the organic vs. the aqueous phase gives the free energy of transfer to water. Thus a negative hydrophathy (or free energy of transfer to water) indicates a tendency to seek the aqueous phase, and vice versa. In order to reduce “noise,” hydrophathy values are actually averages for about 20 amino acid residues. The portion of the hydropathy plot shaded in red in Figure 10-3 indicates the position of the membrane-spanning portion of glycophorin. You may recall that we first encountered hydropathy plots in Chapter 6. You should compare Figure 9-21 to Figure 6-35, a hydropathic index plot for chymotrypsin, a globular protein. Note the fairly random distribution of hydropathic indices along the chymotrypsin, which lacks the shaded region in Figure 9-21. What would a hydropathic index plot for Bacteriorhodopsin, which contains seven membrane-spanning alpha helical segments, look like (see below)?
Another example of a membrane protein is bacteriorhodopsin, a 247-residue protein consisting of a single polypeptide chain. The membrane-spanning domain of Bacteriorhodopsin consists of seven alpha helical segments (See Figure 9-22). Bacteriorhodopsin is involved in a transduction event in which absorbed light is converted to the chemical energy of ATP. The protein is essentially a light-driven proton pump. The light-absorbing pigment is retinal, derived from vitamin A, and is the same light-sensitive element in vision. The structure of retinal, an isoprenoid, is shown below:

![Retinal structure](image)

If the CHO group is reduced to CH₂OH, the resulting form is retinol, or vitamin A, which is derived from beta carotene. Notice the presence of a cis double bond between carbons 11 and 12. All the others (excluding that in the ring) are trans. The absorption of light induces a cis to trans conversion, resulting in a conformational change in the protein to which retinal is bound that leads to the pumping of protons in the case of bacteriorhodopsin and vision in the case of rhodopsin (in our eyes). In bacteriorhodopsin, retinal is bound to a lysine residue via a Schiff’s base.

Figure 9-24 (a) (see page 256) illustrates the transmembrane domain of consisting of an
antiparallel beta sheet rolled up into a barrel (beta barrel, p. 256). Note that this arrangement, as well as alpha helices, satisfies the hydrogen-bonding requirement of transmembrane segments of membrane proteins. In this case hydrogen bonds form between strands. Beta barrels occur in porins, channel-forming proteins in the outer membrane of gram-negative bacteria, and are also found in the outer membranes of eukaryotic mitochondria.

Lipid-Linked Proteins

We’ve seen in previous chapters that carbohydrate can be covalently linked to proteins (via O- or N-linkages) to proteins to form glycoproteins, and that carbohydrate can be covalently linked to lipids to form glycolipids, such as in gangliosides (O-linkage). Lipids can also be covalently linked to proteins to form lipid-linked proteins (as opposed to lipoproteins, which will be discussed later in the chapter). Cysteine residues are involved in attachment of lipids derived from isoprene units (via thioether linkages) to form prenylated proteins, and also in the attachment of fatty acids (either myristate, 14:0, or palmitate, 16:0) via amide (myristate) or thioester (palmitate) linkages.
A third type of lipid-linked proteins is glycosylphosphatidylinositol (GPI) proteins. As the name implies, GPI proteins contain carbohydrate (Figure 9-25) and thus occur exclusively on the exterior of plasma membranes (whereas fatty acid linked proteins, for example, are typically found in subcellular compartments).
Membrane Structure

The technique of freeze-fracture electron microscopy (EM), in which a membrane is frozen in liquid nitrogen and shattered with a sharp blade, illustrated the bilayer nature of membranes.

EM studies also revealed that the interior face of each monolayer (leaflet) is studded with globular particles that are integral membrane proteins.

The fluid mosaic model of a plasma membrane was proposed by Singer and Nicholson in 1972, and is illustrated in Figure 9-26.
This model incorporates several features we’ve already talked about, including the presence of peripheral and integral proteins, a fluid phospholipid core that allows integral proteins to float like icebergs on the two-dimensional phospholipid “sea,” carbohydrate attached either to protein or lipid on the outer leaflet, cholesterol penetrating the bilayer in order to stabilize it, etc. A number of clever experiments have been conducted to illustrate the fluidity of the membrane and its effect on lateral diffusion. Photobleaching, for example, consists of attaching a fluorescent group to a membrane component, then focusing an intense laser beam on a small portion of the membrane, thereby effectively “bleaching” the fluorescence in that area. The observation that fluorescence is quickly recovered in the bleached area indicates rapid lateral diffusion of labeled components back into the bleached area (Figure see page 261)

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**Membrane Skeleton**

Red cell, or erythrocyte, membranes are often used for studies of membrane structure and composition because they are easily isolated and are fairly simple systems. Membranes, or ghosts (so called because of their pale appearance) are easily formed by osmotic lysis. Intact red cells are in the form of a biconcave disk (Figure 9-31), which optimizes their oxygen-carrying capacity. They must be fluid and easily deformable in order to squeeze through small capillaries. These properties are possible because of the membrane skeleton. Prominent features are:
**Spectrin**, comprised of two similar polypeptide chains, constitutes about 75% of the membrane skeleton, and forms a protein meshwork that underlies the inner leaflet (Figure 9-31). Spectrin is anchored to the membrane by its attachment to **ankyrin**, which in turn is bound to an integral membrane protein that functions as an anion channel (CO\(_2\) enters the cell via this channel as HCO\(_3^-\) and is carried by the blood to the lungs (Bohr effect)). This figure also explains why integral membrane proteins exhibit different degrees of mobility within the membrane. According to the **gates and fences** model, some integral proteins are firmly attached to proteins comprising the membrane skeleton, or cytoskeleton, or may be trapped within the spaces by those proteins.

**Lipid Asymmetry**

As we’ve seen, membranes are asymmetric. For example, glycoproteins and glycolipids are typically located on the exterior of the membrane. Studies with phospholipases have shown that there is also considerably asymmetry with respect to composition. Figure 9-33, for example, indicates that the outer leaflet of the erythrocyte membrane consists largely of PC and sphingomyelin, whereas the inner leaflet is richer in PE and the acidic PS. Recall that PS is an acidic phospholipid, hence has a net negative charge on its headgroup, thus contributing to the membrane potential.
In eukaryotes, phospholipids are synthesized in the ER. The very slow transverse diffusion to the outer leaflet is increased about 5 orders of magnitude relative to synthetic bilayers, with the aid of flipases (facilitated diffusion), or via PL translocases (active transport). The action of flipases leads to an equilibrium distribution of PL across the membrane, whereas the latter can move a phospholipid across a bilayer against a concentration gradient via the action of ATP hydrolysis.

In addition to exhibiting transverse asymmetry, as discussed above, membranes are also laterally organized, thus possess lateral asymmetry with regard to both lipid and protein composition. Such asymmetry may be manifested as either domains or microdomains. Divalent cations, such as Ca++, which has been known to cause phospholipid segregation in synthetic liposomes comprised of different phospholipids, including acidic phospholipids such as PS, may cause clustering of these lipids in biological membranes.

One type of microdomain is a lipid raft, appears to consist of closely packed glycosphingolipids and cholesterol. Recall that glycosphingolipids occur on the external leaflet of plasma membranes. The presence of cholesterol is to fill voids between the acyl chains of the glycosphingolipids caused by their large head groups (glycosphingolipids by themselves cannot form bilayers for this reason). This arrangement results in a more ordered, or crystalline, region within the membrane, and these rafts may diffuse laterally within the membrane. The rafts may function as platforms for the assembly of complex intercellular signalling systems. Several viruses, including influenza, measles Ebola and HIV localize to them.

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Problems: 3, 4, 8, 9, 10, 11, 13, 16