CHAPTER 17

AGGREGATION OF AMPHIPHILIC MOLECULES INTO MICELLES, BILAYERS, VESICLES AND BIOLOGICAL MEMBRANES

17.1 INTRODUCTION: EQUILIBRIUM CONSIDERATIONS OF AMPHIPHILIC STRUCTURES

Amphiphilic molecules such as surfactants, lipids, copolymers and proteins can associate into a variety of structures in aqueous solutions (Fig. 16.1), which can transform from one to another when the solution conditions are changed, for example, the electrolyte concentration or pH. To understand these structural aspects one requires an understanding not only of the thermodynamics of self-assembly (discussed in Chapter 16) but also of the forces between the amphiphilic molecules *within* the aggregates and how these, too, are affected by solution conditions. These two factors (thermodynamics and intra-aggregate forces), together with the strength of the inter-aggregate forces *between* aggregates in more concentrated systems, determine the equilibrium structures formed.

In this chapter we shall investigate the interaction forces between amphiphilic molecules within aggregates in more detail, and we shall see how these naturally lead to *molecular packing* considerations in determining which structures are formed. In the following chapter we consider the forces between these structures and their consequences for the equilibrium phase state of the whole system.

Before proceeding it is worth clarifying what one means by 'equilibrium structures', especially with reference to the Gibbs phase rule. Amphiphilic structures can be hard and solid-like, but they are more often soft or *fluid-like*, with the molecules in constant thermal motion within each aggregate—twisting, turning and bobbing in and out of the surface. Thus, unlike monodisperse colloidal particles, these structures have no definite size or shape, but only a *distribution* about some mean value. In Section 16.7 we saw that this distribution can sometimes be very broad.

Further, we also saw that it is possible for the equilibrium distribution to peak at more than one value of N. Thus, in principle, small aggregates such as micelles can be in thermodynamic equilibrium with large aggregates such as vesicles, all within the same one-phase system. While it may appear that a large vesicle or liposome, being a macroscopic structure, should be considered as a separate phase, this is strictly not so. The sizes of the structures play no role in the thermodynamic definition of what constitutes a single phase, which merely requires that the properties be uniform throughout the phase. Thus, in principle, structures may be very large and macroscopic and yet not constitute a separate phase if their number density in solution (or space) remains uniform throughout the whole system which, of course, must be even larger.

Genuine two-phase and three-phase systems can also occur, where monomers, micelles, vesicles or liposomes separate out into distinct phases in equilibrium with each other. However, such phase separations can take a long time to reach equilibrium, so that it is often difficult to identify experimentally the true thermodynamic state of an amphiphilic system, let alone study the structure and dynamics of the aggregates themselves.

17.2 OPTIMAL HEADGROUP AREA

The major forces that govern the self-assembly of amphiphiles into well-defined structures such as micelles and bilayers derive from the hydrophobic attraction at the hydrocarbon–water interface, which induces the molecules to associate, and the hydrophilic, ionic or steric repulsion of the headgroups, which imposes the opposite requirement that they remain in contact with water. These two interactions compete to give rise to the idea of two 'opposing forces' (Tanford, 1980) acting mainly in the interfacial region: the one tending to decrease and the other tending to increase the interfacial area a per molecule (the effective headgroup area) exposed to the aqueous phase (Fig. 17.1).

The attractive interaction arises mainly from the hydrophobic or interfacial tension forces which act at the essentially fluid hydrocarbon–water interface. This interaction may be represented by a positive interfacial free energy per unit area characteristic of the hydrocarbon–water interface of $\gamma \approx 50\ \text{mJ m}^{-2}$, though as discussed in Section 16.6 there are indications that at a hydrophilic headgroup–water interface this value may be much reduced and closer to $\gamma \approx 20\ \text{mJ m}^{-2}$ (Parsegian, 1966; Jönsson and Wennerström, 1981; see also Table 16.2). In the case of biological lipids, about half of these usually contain one or more unsaturated C=C bonds and these have much lower interfacial

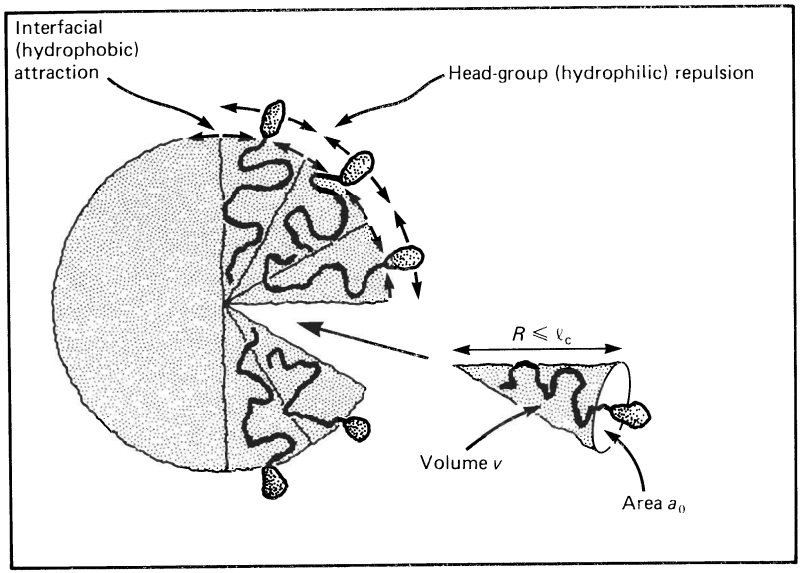


Fig. 17.1. The hydrocarbon interiors in both micelles and bilayers are normally in the fluid state (Shinitzky *et al.*, 1971; Lindblom and Wennerström, 1977). Repulsive headgroup forces and attractive hydrophobic interfacial forces determine the optimum headgroup area a_0 at which μ_N^0 is a minimum (see Fig. 17.2). The chain volume v and chain length l_c set limits on how the fluid chains can pack together, on average, inside an aggregate. Thus, the mean molecular conformation depends on a_0, v and l_c

energies with water than the unsaturated homologues (cf. Table 15.1 where γ_{12} falls from 52 to 19 mJ m^{-2} on replacing a C—C bond by a C=C bond in octadecane).

Thus the attractive interfacial free energy contribution to μ_N^0 may be simply written as γa, where γ is expected to lie between 20 and 50 mJ m^{-2}. This simple expression is modified when account is taken of the non-purely liquid-like nature of the hydrocarbon chains, but it serves as a good first approximation.

The repulsive contributions are still too complex and difficult to formulate explicitly (Israelachvili *et al.*, 1980a; Puvvada and Blanckstein, 1990). Between mobile hydrophilic headgroups these include a steric contribution, a hydration force contribution and an electrostatic double-layer contribution if the headgroups are charged (Payens, 1955; Forsyth *et al.*, 1977). Luckily, these separate contributions do not have to be known explicitly. This is because—as in the two-dimensional van der Waals equation of state—we expect the first term in any energy expansion to be inversely proportional to the surface area occupied per headgroup a (cf. pressure $\propto 1/a^2$ in Eq. (2.29)).

The total interfacial free energy per molecule in an aggregate may therefore be written, to first order, as

$$\mu_N^0 = \gamma a + K/a, \tag{17.1}$$

where K is a constant. We shall initially assume that both these forces act in the same plane at the hydrophobic–hydrophilic interface (Fig. 17.1). The minimum energy is therefore given when $\partial \mu_N^0 / \partial a = 0$, that is,

$$\mu_N^0(\min) = 2\gamma a_0, \qquad a_0 = \sqrt{K/\gamma}. \tag{17.2}$$

a_0 will be referred to as the *optimal surface area* per molecule, defined at the hydrocarbon - water interface. The interfacial energy per molecule, Eq. (17.1) may now be expressed in the more convenient form

$$\mu_N^0 = 2\gamma a_0 + \frac{\gamma}{a}(a - a_0)^2 \tag{17.3}$$

in which the unknown constant K has been eliminated, so that μ_N^0 as a function of a is now in terms of the two known or measurable parameters, γ and a_0.

We see therefore how the concept of opposing forces leads to the notion of an optimal area per headgroup at which the total interaction energy per lipid molecule is a minimum (Fig. 17.2). Moreover, for truly fluid hydrocarbon chains, the optimal area should not depend strongly on the chain length or

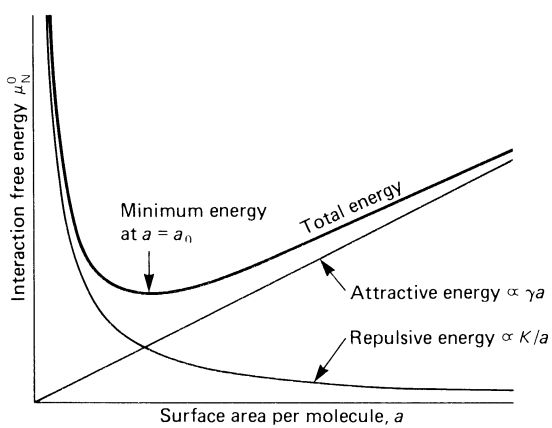


Fig. 17.2. Optimal headgroup area a_0 at which the opposing forces of headgroup repulsion and interfacial (hydrophobic) attraction are balanced.

on the number of chains, as is indeed found experimentally (Gallot and Skoulios, 1966; Reiss-Husson, 1967; Lewis and Engelman, 1983b).

The above equations, while crude, contain the essential features of interlipid interactions in micelles, bilayers and membranes. They imply that, to a first approximation, the interaction energy between lipids has a minimum at a certain headgroup area a_0, about which the energy varies parabolically (i.e., elastically). The equations ignore three second-order but nevertheless important effects, some of which are discussed later: (i) specific headgroup interactions such as ionic bridging, (ii) specific chain–chain interactions (since the hydrocarbon chains are never perfectly fluid), and (iii) the effect of surface curvature on μ_N^0.

17.3 GEOMETRIC PACKING CONSIDERATIONS

Having established the equations that adequately describe the interactions between amphiphilic molecules within an aggregate, we have yet to establish the most favoured structures. The geometry or 'packing' properties of the molecules now enter the picture. These depend on their optimal area a_0, the volume v of their hydrocarbon chain or chains, which will be assumed to be fluid and incompressible, and the maximum effective length that the chains can assume. We shall call this the *critical chain length*, l_c. This length sets a limit on how far the chains can extend; smaller extensions are allowed but further extensions are not, these being prevented by a sharp rise in the interaction energy. The critical length l_c is a semiempirical parameter, since it represents a somewhat vague cutoff distance beyond which hydrocarbon chains can no longer be considered as fluid. However, as may be expected, it is of the same order as, though somewhat less than, the fully extended molecular length of the chains l_{max} (Tanford, 1973, 1980; Israelachvili *et al.*, 1976, 1977). According to Tanford, for a saturated hydrocarbon chain with n carbon atoms:

$$l_c \leqslant l_{max} \approx (0.154 + 0.1265n)\ \text{nm} \tag{17.4}$$

and

$$v \approx (27.4 + 26.9n) \times 10^{-3}\ \text{nm}^3. \tag{17.5}$$

Note that for large n, $v/l_c \approx 0.21\ \text{nm}^2 \approx$ constant, which is close to the minimum cross-sectional area that a hydrocarbon chain can have.

Once the optimal surface area a_0, hydrocarbon chain volume v and critical length l_c are specified for a given molecule—all these being measurable or estimable—one may ascertain which structures the molecules can pack into consistent with these geometric constraints. It turns out that these can be satisfied by a great variety of different structures. However, since μ_N^0 will be roughly the same for all these structures (since a_0 is the same) entropy will favour the structure with the smallest aggregation number, say, at $N = M$, and this structure is unique! Larger structures will be entropically unfavoured, while smaller structures, where packing constraints force the surface area a to increase above a_0, will be energetically unfavoured.

It has been shown previously (Israelachvili *et al.*, 1976) that for lipids of optimal area a_0, hydrocarbon volume v and critical chain length l_c, the value of the dimensionless *packing parameter* or *shape factor*, $v/a_0 l_c$, will determine whether they will form spherical micelles ($v/a_0 l_c < \frac{1}{3}$), non-spherical micelles ($\frac{1}{3} < v/a_0 l_c < \frac{1}{2}$), vesicles or bilayers ($\frac{1}{2} < v/a_0 l_c < 1$), or 'inverted' structures ($v/a_0 l_c > 1$). Each of these structures corresponds to the minimum-sized aggregate in which all the lipids have minimum free energy. These structures will now be described in turn.

17.4 SPHERICAL MICELLES

For molecules to assemble into spherical micelles, their optimal surface area a_0 must be sufficiently large and their hydrocarbon volume v sufficiently small that the radius of the micelle R will not exceed the critical chain length l_c. From simple geometry we have, for a spherical micelle of radius R and mean aggregation number M (Fig. 17.1),

$$M = 4\pi R^2/a_0 = 4\pi R^3/3v, \tag{17.6}$$

namely,

$$R = 3v/a_0, \tag{17.7}$$

so that only for

$$\frac{v}{a_0 l_c} < \tfrac{1}{3} \tag{17.8}$$

will the amphiphiles be able to pack into a spherical micelle with their headgroup areas equal to a_0 and with the micelle radius R not exceeding l_c.

An example of such micelle-forming amphiphiles is the 12-carbon chain

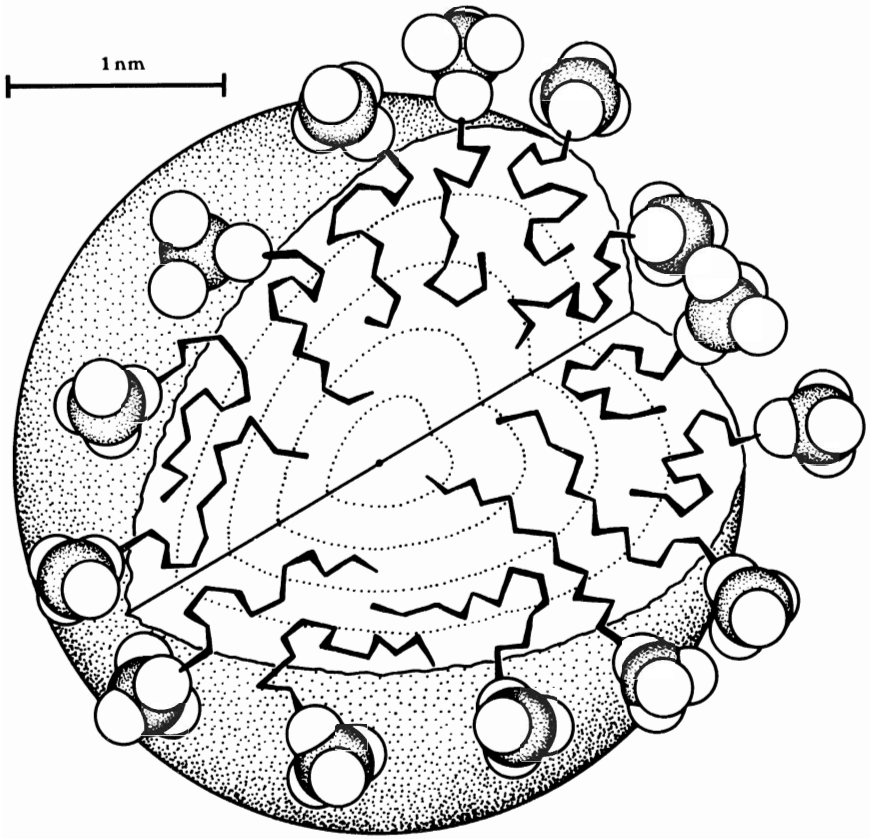


Fig. 17.3. A sodium dodecylsulphate (SDS) micelle drawn to scale. The micelle contains 60 sodium dodecylsulphate molecules. The hydrocarbon chains pack at liquid hydrocarbon density in the core where they are almost as disordered as in the bulk liquid state. Each of the five spherical shells contains approximately the correct number of chain segments to ensure even chain packing density throughout. Note that all segments of the chain spend an appreciable proportion of time near the micelle surface. Thus, even though the core is almost completely devoid of water each segment samples the hydrophilic environment. Drawing based on calculations by Gruen (1981) and Gruen and de Lacey (1984).

sodium dodecyl sulphate surfactant (SDS) in water (Fig. 17.3) where experimentally $M \approx 74$ (Cabane *et al.*, 1985). Putting $n = 12$ into Eq. (17.5) gives $v = 0.3502\ \text{nm}^3$. Equation (17.6) then gives $a_0 \approx 0.57\ \text{nm}^2$, and Eq. (17.7) gives for the optimal micelle radius: $R \approx 1.84$ nm. Now for a 12-carbon chain, Eq. (17.4) gives $l_c \approx 1.67$ nm, which is 0.17 nm short of the required (optimal) radius. Thus, for SDS micelles in water, $v/a_0 l_c \approx 0.37$, which means that they just cannot pack into spheres and so must be slightly non-spherical.

□

● WORKED EXAMPLE ●

Question: Below what aggregation number will SDS micelles in water be spherical and how could this be achieved in practice.

Answer: After some thought or algebra, using Eqs (17.6)–(17.8), it becomes clear that the answer is simply

$$M = \frac{4\pi l_c^3}{3v} = \frac{4\pi[(0.154 + 0.1265 \times 12)10^{-9}]^3}{3[(27.4 + 26.9 \times 12)10^{-30}]} = 56.$$

We have seen that experimentally, $M = 74$ and $v/a_0 l_c \approx 0.37$ for SDS in water. Since v and l_c are fixed, the only way to reduce $v/a_0 l_c$ to the required value of 0.33 is to raise a_0 by about 10%. In practice, this could be achieved by raising the pH of the solution. This would increase the degree of ionization of the negatively charged headgroups which increases the repulsion between them (see Problem 12.7), resulting in an increase in a_0. The required value of a_0 is given by Eq. (17.8) as $3v/l_c = 0.63\ \text{nm}^2$. If the surfactant were cationic, we would decrease the pH. □

The mean size of spherical micelles is relatively insensitive to the surfactant concentration above the CMC, and the micelles are fairly monodisperse. The standard deviation σ in the aggregation number about the mean (at $N \approx M$ where $a = a_0$) may be obtained by first noting that μ_N^0 of Eq. (17.3) can be written as

$$\mu_N^0 = \mu_M^0 + \frac{\gamma}{a}(a - a_0)^2. \tag{17.9}$$

Now for a spherical micelle, we have $N = 4\pi R^2/a = 4\pi R^3/3v = 36\pi v^2/a^3$. Equation (17.9) may therefore be expressed as

$$\mu_N^0 - \mu_M^0 = \Lambda(N - M)^2, \qquad \text{where } \Lambda = \gamma a_0/9M^2. \tag{17.10}$$

Substituting this value for Λ into Eq. (16.30) gives

$$\sigma = \sqrt{(9kT/2\gamma a_0)M}. \tag{17.11}$$

Typically, for γ lying in the range $20–50\ \text{mJ m}^{-2}$ and $a_0 \approx 0.60\ \text{nm}^2$, we therefore expect

$$\sigma \approx \sqrt{M}, \tag{17.12}$$

for example, for $M \approx 60$, $\sigma \approx 8$. Aniansson *et al.* (1976) found that for a variety of sodium alkyl sulphate micelles, the value of $\sigma/\sqrt{M}$ varies between 1 and 2. The distribution or spread about M is thus fairly narrow but by no means sharp, as illustrated in Fig. 16.6.

17.5 NON-SPHERICAL AND CYLINDRICAL MICELLES

Most lipids that form spherical micelles have charged headgroups since this leads to large headgroup areas a_0. Addition of salt partially screens the electrostatic inter-headgroup repulsion and thereby reduces a_0. Those lipids that possess smaller headgroup areas such that $\frac{1}{3} < v/a_0 l_c < \frac{1}{2}$ cannot pack into spherical micelles but can form cylindrical (rod-like) micelles. Falling into this category are single-chained lipids possessing charged headgroups in high salt (e.g., SDS, CTAB) or those possessing uncharged, non-ionic or zwitterionic headgroups (e.g., $C_{12}E_5$, lysolecithin).

As discussed in Section 16.7 rod-like aggregates must have very unusual properties: they are large and polydisperse, and their mean aggregation number is very sensitive to the total lipid concentration C. According to Eq. (16.24) above the CMC, their mean aggregation number should increase proportionally to $\sqrt{C}$, which is indeed found to be the case experimentally (Mazer *et al.*, 1976; Missel *et al.*, 1980).

It is important to note that the unusual properties of cylindrical micelles are due entirely to 'end effects': at each end the lipids are forced to pack into hemispherical caps with a headgroup area a determined by $v/al_c = \frac{1}{3}$, so that $a > a_0$ since $v/a_0 l_c > \frac{1}{3}$. The unfavourable energy of these end lipids determines the magnitude of the interaction parameter α in Eq. (16.24), which increases as a_0 decreases. Since $\langle N \rangle = 2\sqrt{Ce^{\alpha}}$ it is not surprising that the growth of cylindrical micelles is very sensitive to changes in temperature, chain length, and—for ionic lipids—to ionic strength (Missel *et al.*, 1980, 1983; Malliaris *et al.*, 1985; Lin *et al.*, 1990; Wennerström and Lindman, 1979). For example, their sensitivity to increasing ionic strength arises from its effect on decreasing a_0, which increases α. As might be expected the mean aggregation number increases dramatically with increased ionic strength. Thus, the aggregation number of SDS micelles in 0.6 M NaCl is $\sim$1000 compared to $\sim$60 in water. The unfavourable end energy of cylindrical micelles may be eliminated if the two ends join, thereby forming a toroidal micelle (the two-dimensional analogue of a vesicle). However, toroidal micelles have so far not been observed.

17.6 BILAYERS

Lipids that form bilayers are those that cannot pack into micellar structures due to their small headgroup area a_0 or because their hydrocarbon chains are too bulky to fit into such small aggregates while maintaining the surface area at its optimal value. For bilayer-forming lipids, the value of $v/a_0 l_c$ must lie close to 1, and this requires that for the same headgroup area a_0 and chain length l_c, their hydrocarbon volume v must be about twice that of micelle-forming lipids (for which $v/a_0 l_c$ is in the range $\frac{1}{3}$ to $\frac{1}{2}$). Therefore, lipids with *two* chains are likely to form bilayers, and indeed most of them do. For example, single-chained lysolecithins form small but non-spherical micelles while lecithins with two alkyl chains, such as di-C_{14} and di-C_{16} lecithins, form bilayers (Fig. 17.4). Similarly, CTAB forms micelles while the double-chained analogue forms bilayers.

The doubling of the chains also affects other aggregate properties, both static and dynamic. First, it increases the hydrophobicity of the lipids, which in turn drastically lowers their CMC: compare the CMCs of common micelle-forming lipids (10^{-2}–10^{-5} M) with those of bilayer-forming lipids (10^{-6}–10^{-10} M). We shall return to comment on the biological significance of such low CMCs later. Second, it increases the lifetimes or 'residence times', τ_R, of the molecules within aggregates. Self-assembled structures are usually highly dynamic, with the molecules in constant thermal motion within the aggregates as well as exchanging with monomers in the bulk solution (Pfeiffer *et al.*, 1989). This diffusive exchange may be understood as an activation process, whereby a certain activation energy ΔE has to be surmounted before a molecule can escape from the micelle or bilayer into the bulk solution. Consider the molecules within a bilayer to be jumping about with some characteristic 'collision time', τ_0. Since the probability of a molecule leaving the bilayer each time it hits the interface is $e^{-\Delta E/kT}$, the mean lifetime of a molecule is therefore $\tau_0/e^{-\Delta E/kT}$. After a little thought it becomes clear that ΔE is essentially the same as $(\mu_1^0 - \mu_N^0)$ in Eq. (16.13), which allows us finally to express the residence time in the simple form

$$\tau_R = \tau_0/e^{-\Delta E/kT} \approx 55\tau_0/\text{CMC}, \tag{17.13}$$

where the CMC is in units of M. Typical motional correlation times for amphiphiles in micelles and bilayers are in the range $\tau_0 = 10^{-9}$–10^{-7} s. Thus for micelles and bilayers we find

$$\tau_R \text{ (micelles)} \sim 55 \times 10^{-9}/10^{-3} \sim 10^{-4} \text{ s},$$

$$\tau_R \text{ (bilayers)} \sim 55 \times 10^{-7}/10^{-10} \sim 10^{+4} \text{ s}.$$

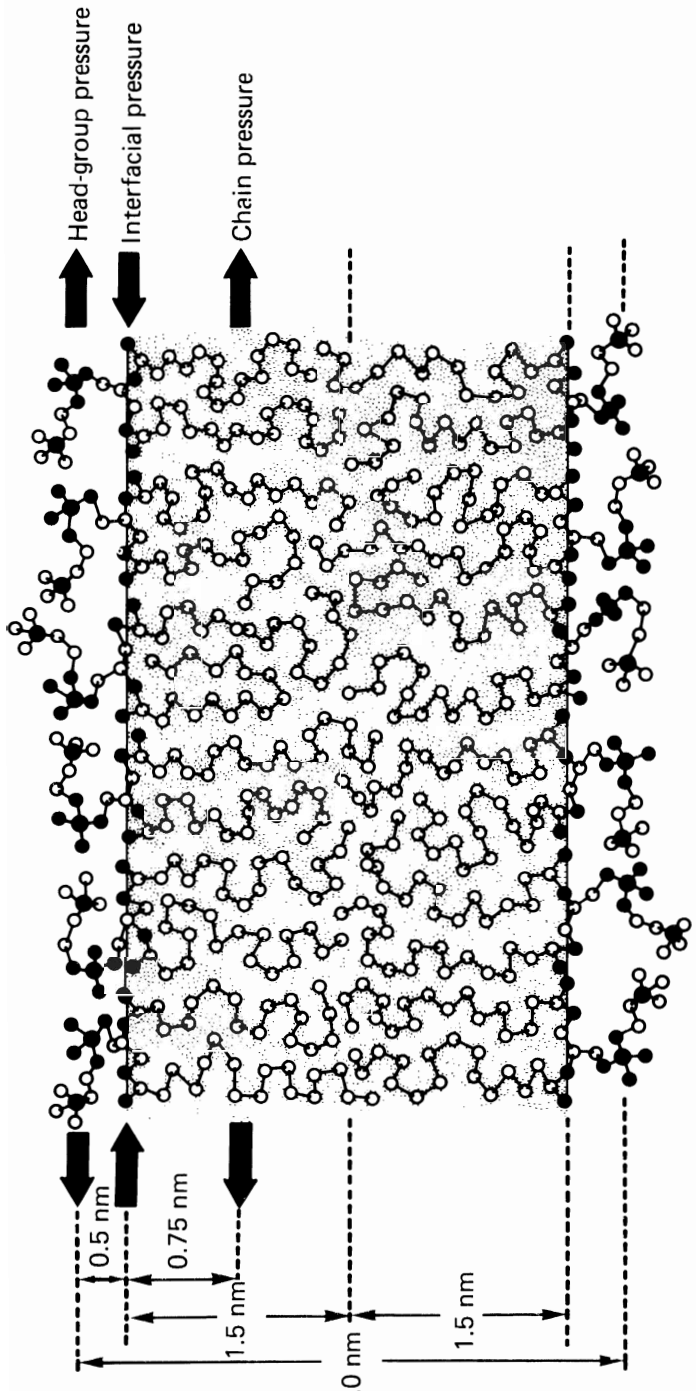


Fig. 17.4. Lecithin bilayer drawn to scale. The lipid bilayer is the basic structure of biological membranes, and most membrane lipids contain two hydrocarbon chains. The lipids diffuse rapidly in the plane of the bilayer, covering a distance of about 1 μm in 1 s. They also cross the bilayer from one side to the other ('flip-flop'), as well as exchange with lipids in the solution, but at much slower rates, of the order of hours (cf. the lifetime of single-chained surfactants in micelles of 10^{-5} to 10^{-3} s). (From Israelachvili *et al.*, 1980a.)

These values are typical of those measured (Wennerström and Lindman, 1979; Wimley and Thompson, 1990). Furthermore, from the measured CMCs of lipids (Table 16.2) Eq. (17.13) suggests that exchange rates should fall by a factor of about 4–10 per two CH_2 groups added to the chains, in good agreement with measured values of about 8 for phospholipids (Homan and Pownall, 1988). Note, however, that residence times depend on the individual molecules and not on the structures. Thus, when a double-chained lipid bilayer hosts a single-chained surfactant molecule, the residence time of the guest lipid will be much shorter than that of the host lipids.

Another dynamic effect in bilayers is transbilayer lipid exchange, or the 'flip-flop' of molecules from one side to the other. This may also be viewed as a diffusive exchange process, except that the energy ΔE in Eq. (17.13) now refers to the energy needed to put the hydrophilic headgroup into the hydrophobic region of the bilayer. However, other mechanisms for flip-flop have also been proposed such as the diffusion of molecules around the walls of transient pores (see Fig. 17.9 and Problem 17.9). Flip-flop energies are generally higher than those for lipid exchange so that flip-flop times are longer and typically range from minutes to days (10^2–10^5 s) and, unlike interbilayer exchange, depend more on the headgroup than on the chains (Homan and Pownall, 1988; Wimley and Thompson, 1990).

When a bilayer is stretched from its equilibrium state it expands elastically. For fluid bilayers the area compressibility modulus k_a may be readily estimated from Eq. (17.3) since by definition

$$\text{elastic energy} = \tfrac{1}{2}k_a(a - a_0)^2/a, \tag{17.14}$$

which gives

$$k_a \approx 2\gamma \text{ per monolayer}, \tag{17.15a}$$

$$\approx 4\gamma \text{ per bilayer}. \tag{17.15b}$$

Assuming $\gamma \approx 20$–$50\ \text{mJ m}^{-2}$, we therefore expect $k_a \approx 4\gamma \approx 80$–$200\ \text{mJ m}^{-2}$ for bilayers. This estimate compares well with measured values on fluid lipid bilayers and free biological cell membranes which range from 100–230 mJ m^{-2} (Kwok and Evans, 1981; Evans and Rawicz, 1990; Marsh, 1990). Bilayers also have an elastic bending or curvature modulus, k_b, which affects the energy of curved bilayers and vesicles (Section 17.9). Both the area and bending moduli play an important role in determining the thermal fluctuation forces between bilayers, discussed in Section 18.6.

So far, it has been implicitly assumed that the hydrocarbon chains in micelles and bilayers are in the fluid state. At room temperature this is the case for most micelle-forming single-chained surfactants, as well as for

TABLE 17.1 Chain melting (phase transition) temperatures, T_c, of some common double-chained lipid bilayers in water (at pH 7) in order of increasing T_c

Lipid (giving number of carbons per chain)	Headgroup type[a] and chain melting temperature,[b] T_c (°C)				Melting point of *n*-alkane with same number of carbon atoms
	PC	PG^-	PS^-	PE	
Saturated					
Dilauroyl (12)	−2	0	13	30	−9.6
Dimyristoyl (14)	23	24	36	49	5.9
Dipalmitoyl (16)	41	41	52	64	18.2
Distearoyl (18)	55	55	68	74	28.2
Unsaturated (cis)					
Dioleoyl (18)	−22	−18	−7	−16	−30

[a] PC: phosphatidylcholine (zwitterionic); PG^-: phosphatidylglycerol (negatively charged); PS^-: phosphatidylserine (negatively charged); PE: phosphatidylethanolamine (zwitterionic).
[b] Compiled from Cevc and Marsh (1987) and Marsh (1990).

bilayer-forming double-chained lipids having less than 14 carbons per chain. Unsaturated and branched chained lipids remain in the fluid state down to much lower temperatures. Some typical lipid chain melting temperatures, T_c, are given in Table 17.1. We see that these are somewhat above the melting points of the corresponding *n*-alkane, i.e., without the headgroup. At temperatures below T_c, bilayers cease to be fluid-like: the elastic moduli increase, lateral diffusion and flip-flop rates fall, etc. However, bilayers below T_c do not always freeze into crystalline solids, but often retain some of their fluid-like properties, for example, the headgroups may still be highly mobile even though the chains are not. Such bilayers are usually referred to as being in the *gel* state. For an excellent book covering all aspects of the physical properties of lipid bilayers see *Phospholipid Bilayers* by Cevc and Marsh (1987), and the *CRC Handbook of Lipid Bilayers* (Marsh, 1990) for factual details.

17.7 VESICLES

Under certain conditions it becomes more favourable for closed spherical bilayers (vesicles) to form rather than infinite planar bilayers. This arises since in a closed bilayer the energetically unfavourable edges are eliminated at a finite, rather than infinite, aggregation number, which is also entropically

favoured. Thus, so long as the lipids in a curved bilayer can maintain their areas at their optimal value, vesicles should be the preferred structures. What then determines the radii of vesicles? First, let us note that if $v/a_0 l_c = 1$, only planar bilayers will form. For a bilayer to curve, the lipids in the outer monolayer must be able to pack, on average, into truncated cones. This requires that $v/a_0 l_c < 1$. Simple geometric considerations show (Israelachvili *et al.*, 1976) that for $\frac{1}{2} < v/a_0 l_c < 1$, the radius of the smallest vesicle that may be formed without forcing the headgroup area a in the outer monolayer to exceed a_0 is given by

$$R_c \approx l_c \left[\frac{3 + \sqrt{3(4v/a_0 l_c - 1)}}{6(1 - v/a_0 l_c)} \right] \approx \frac{l_c}{(1 - v/a_0 l_c)} \qquad (17.16)$$

which is the *critical radius* below which a bilayer cannot curve without introducing unfavourable packing strains on the lipids. So long as a vesicle's radius does not fall below R_c the lipids in both the inner and outer monolayers can pack with their surface areas at the optimal value a_0 and with the two hydrocarbon chain regions not exceeding l_c. For $R < R_c$, a must exceed a_0 in the outer monolayer and such vesicles are energetically unfavoured, while for $R > R_c$ the vesicles are entropically unfavoured. For a vesicle of radius R_c and bilayer hydrocarbon thickness $t \approx 2v/a_0$, the aggregation number is

$$N \approx 4\pi[R_c^2 + (R_c - t)^2]/a_0. \qquad (17.17)$$

As an example, we may apply the above equations to the much-studied egg lecithin vesicles for which $a_0 \approx 0.717\,\text{nm}^2$ and $v \approx 1.063\,\text{nm}^3$. Taking $l_c \approx 1.75$ nm (i.e., $v/a_0 l_c \approx 0.85$), Eqs (17.16) and (17.17) then yield $R_c \approx 11$ nm, $N \approx 3000$, $t \approx 3.0$ nm, and an outside-to-inside lipid ratio of $R_c^2/(R_c - t)^2 \approx 1.9$, all in good agreement with measured values.

Many people believe that vesicles represent the prototypes of early living cells, and it has now been demonstrated that certain biological lipids as well as synthetic surfactants and mixtures can spontaneously self-assemble into stable monodisperse vesicles (Brunner *et al.*, 1976; Gains and Hauser, 1983; Hauser *et al.*, 1983, 1990; Hauser, 1984; Talmon *et al.*, 1983; Kaler *et al.*, 1989; Madani and Kaler, 1990).

Finally, for lipids with very small optimal headgroup areas (e.g., $a_0 < 0.42\,\text{nm}^2$ for double-chained lipids) or with bulky polyunsaturated chains (large v, small l_c), their value of $v/a_0 l_c$ will exceed unity. According to Eq. (17.16), when $v/a_0 l_c > 1$, R_c becomes negative. What this means is that such lipids form 'inverted' micellar structures or precipitate out of solution (e.g., MGDG, unsaturated phosphatidylethanolamines, negatively charged lipids in the presence of Ca^{2+} ions, cholesterol).

17.8 FACTORS AFFECTING CHANGES FROM ONE STRUCTURE TO ANOTHER

We have seen that the geometric packing properties of different lipids may be conveniently expressed in terms of the packing parameter $v/a_0 l_c$ characteristic for each lipid in a given solution environment, the value of which determines the type of aggregate formed. Table 17.2 illustrates the structures formed by some common lipids, and how these can be modified by their ionic environment, temperature, chain unsaturation, etc., as will now be summarized.

(i) *Factors affecting headgroup area.* Lipids with smaller headgroup areas (high $v/a_0 l_c$) form larger vesicles, less-curved bilayers, or inverted micellar phases. For anionic headgroups, this can be brought about by increasing the salt concentration, particularly Ca^{2+}, or lowering the pH. This also has the effect of straightening (condensing) the chains.

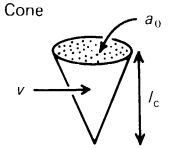
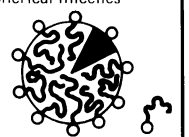
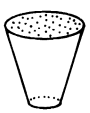
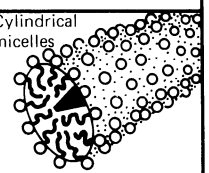
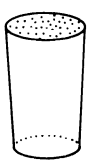
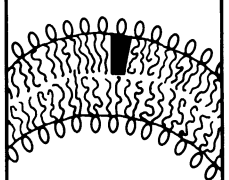
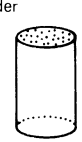
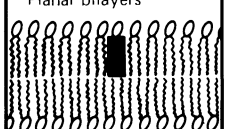
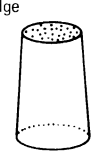
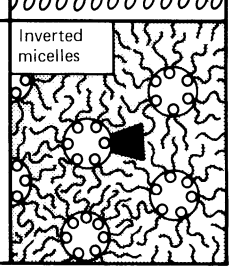
(ii) *Factors affecting chain packing.* Introducing chain branching and unsaturation, particularly of *cis* double bonds, reduces l_c and thus increases $v/a_0 l_c$. Similar effects occur when the effective volume, v, of the chains is increased due to the penetration of organic molecules such as low MW alkanes into the chain regions.

Both the above effects lead to larger vesicles and ultimately to inverted structures. In the case of microemulsions (surfactant/water/oil mixtures), they lead to larger 'oil-in-water' droplets and ultimately to inverted 'water-in-oil' droplets.

(iii) *Effects of temperature T.* Changing the temperature can alter both a_0 and l_c so that these effects are more subtle and generally less well understood. For double-chained lipids in the fluid state ($T > T_c$), increasing T increases the hydrocarbon chain motion involving *trans-gauche* isomerization and thereby reduces their limiting length l_c. If the headgroup area a_0 does not change significantly, as occurs for charged headgroups, the net effect is an increase in $v/a_0 l_c$. However, changing T can also change a_0. For example, the areas of polyoxyethylene headgroups *decrease* with increasing T due to their increased hydrophobicity (Puvvada and Blanckstein, 1990), and this acts to enhance the increase in $v/a_0 l_c$. But the areas of more hydrophilic headgroups usually *increase* with T due to the increased steric repulsion between them, and this acts to decrease $v/a_0 l_c$. Thus, with increasing temperature non-ionic spherical micelles grow in size and become more cylindrical (Kato and Seimiya, 1986; Herrington and Sahi, 1988), while charged micelles shrink (Missel *et al.*, 1980). Zwitterionic micelles appear to behave somewhere in between, and their aggregation number hardly changes with temperature (Malliaris *et al.*, 1985).

(iv) *Lipid mixtures.* When an aggregate is composed of a lipid mixture, so long as the different molecules mix ideally and do not phase-separate, the

TABLE 17.2 Mean (dynamic) packing shapes of lipids and the structures they form

Lipid	Critical packing parameter $v/a_0 l_c$	Critical packing shape	Structures formed
Single-chained lipids (surfactants) with large head-group areas: *SDS in low salt*	$< 1/3$	Cone 	Spherical micelles 
Single-chained lipids with small head-group areas: *SDS and CTAB in high salt, nonionic lipids*	1/3-1/2	Truncated cone 	Cylindrical micelles 
Double-chained lipids with large head-group areas, fluid chains: *Phosphatidyl choline (lecithin), phosphatidyl serine, phosphatidyl glycerol, phosphatidyl inositol, phosphatidic acid, sphingomyelin, DGDG[a], dihexadecyl phosphate, dialkyl dimethyl ammonium salts*	1/2-1	Truncated cone 	Flexible bilayers, vesicles 
Double-chained lipids with small head-group areas, anionic lipids in high salt, saturated frozen chains: *phosphatidyl ethanolamine, phosphatidyl serine + Ca^{2+}*	~1	Cylinder 	Planar bilayers 
Double-chained lipids with small head-group areas, nonionic lipids, poly *(cis)* unsaturated chains, high T: *unsat. phosphatidyl ethanolamine, cardiolipin + Ca^{2+} phosphatidic acid + Ca^{2+} cholesterol, MGDG[b]*	> 1	Inverted truncated cone or wedge 	Inverted micelles 

[a] DGDG, digalactosyl diglyceride, diglucosyl diglyceride.
[b] MGDG, monogalactosyl diglyceride, monoglucosyl diglyceride.

aggregate properties may be treated, in a first approximation, in terms of some mean packing parameter intermediate between those of the individual components (Carnie *et al.*, 1979). The sizes of vesicles may thus be conveniently increased or decreased by adding an appropriate amount of another component whose packing parameter is larger or smaller than that of the host lipid. In the case of microemulsion droplets, their sizes are often modulated in this way by adding a 'cosurfactant'. In other cases, totally new structures may be obtained by a suitable choice of lipid additive. For example, micelle-forming lysolecithin ($v/a_0 l_c < 0.5$) and non-aggregate forming cholesterol ($v/a_0 l_c > 1.0$) mix in certain proportions to form bilayer vesicles ($0.5 < v/a_0 l_c < 1.0$).

The above effects of headgroup size, ionic strength, unsaturation, and temperature are illustrated in Table 17.2 for dilute lipid structures in aqueous solutions. It is worth noting that micelles, bilayers and other self-assembling structures also form in other hydrogen-bonding liquids, such as ethylene glycol, formamide and hydrazine (N_2H_4), but their properties have not been as extensively studied. The main requirement for the solvent appears to be that it have a high interfacial energy with hydrocarbons (see Table 15.1).

17.9 CURVATURE ELASTICITY OF BILAYERS AND MEMBRANES

The above treatment offers a rough and ready recipe for analysing the packing properties of lipid structures, but it is nevertheless incomplete. The only restriction we have so far considered is that the fluid chains cannot extend away from the headgroup further than a certain distance l_c. This leads to an increased energy only for radii below R_c, but for $R > R_c$ there is no curvature dependence because the lipid molecules can now arrange themselves with their areas at the optimal value. However, due to our simplifying assumptions we have missed two other contributions to the energy, which will now be investigated.

(i) *Headgroup repulsion.* The attractive and repulsive interfacial forces that determine a_0 and μ_N^0 in Eq. (17.3) were both assumed to act in the same plane, at the hydrocarbon–water interface, at which the surface area per molecule has been defined. This is likely to be true for the attractive interfacial tension force but not for the headgroup repulsive forces, which are likely to be centred at some finite distance D above the interface as shown in Figs 17.1 and 17.4.

(ii) *Chain repulsion.* It was assumed that hydrocarbon chains are entirely fluid and do not oppose any distortion until they become forced to extend

beyond l_c. In other words, it was assumed that there is no curvature dependence of μ_N^0 due to the non-fluidity of chains. Gruen and de Lacey (1984), inspired by the earlier theory of Marcelja (1974), analysed the energetics of chain packing in both micelles and bilayers and found that the assumption of fluidity in bilayers is much less valid than it is in micelles. They concluded that $l_c \approx l_{max}$ in spherical and cylindrical micelles, but that $l_c \approx 0.7 l_{max}$ in bilayers (cf. the assumed value of $l_c \approx 1.75\,\text{nm} \approx 0.75 l_{max}$ for egg lecithin vesicles in Section 17.7). Further, the restriction of chain freedom in bilayers gives rise to an additional lateral chain pressure acting inside the hydrocarbon region, i.e., at some negative distance $-D$ from the hydrocarbon–water interface (Fig. 17.4).

It can be shown that both the above effects result in an additional curvature dependence of μ_N^0 on R. For bilayers this has the form

$$\Delta E = -2\gamma t D/R^2 = \tfrac{1}{2}k_b/R^2 \text{ per unit area}, \qquad (17.18)$$

where t is the bilayer thickness and D the distance out from the hydrocarbon–water interface where the repulsive forces are centred (Figs 17.1 and 17.4). By expressing the curvature or bending energy as $\frac{1}{2}k_b/R^2$ we adopt a convention where k_b is, by definition, the *bending modulus* of an elastic sheet. Using typical bilayer parameters such as $\gamma = 20\,\text{mJ m}^{-2}$, $t = 3.0\,\text{nm}$ and $D = \pm 0.3\,\text{nm}$, we obtain $k_b = \pm 4\gamma t D \approx \pm 7 \times 10^{-20}\,\text{J}$. This is within the range of (positive) values measured for fluid bilayers: $(2\text{–}20) \times 10^{-20}\,\text{J}$ (Evans and Rawicz, 1990; Marsh, 1990; Abillon and Perez, 1990). However, surfactant *monolayers*, as occur at oil–water interfaces, appear to have much lower bending moduli, of the order of $5 \times 10^{-21}\,\text{J}$ (Safinya *et al.*, 1986).

Note that depending on where the repulsive and attractive forces are located, k_b can be positive or negative. In case (i) above, when the headgroup repulsion dominates, D is positive so that k_b is *negative*. Thus, headgroup repulsion *favours* bending right from the start. In case (ii) where the chain repulsion dominates, D is negative and k_b is therefore positive. Thus, chain repulsion acts to oppose bending. Let us consider these two cases in more detail (see also Israelachvili *et al.*, 1976, 1980a, 1987c).

Positive curvature modulus ($k_b > 0$)

The interaction energy per vesicle of large radius R ($R > R_c$) and aggregation number N now becomes

$$N\mu_N^0 = N\mu_\infty^0 + (\tfrac{1}{2}k_b/R^2)4\pi R^2 = N\mu_\infty^0 + 2\pi k_b \qquad (17.19)$$

and thus

$$\mu_N^0 = \mu_\infty^0 + 2\pi k_b/N \qquad (17.20)$$

which is the same as Eq. (16.6) with $\alpha = 2\pi k_b/kT$. Thus, if C is the total lipid concentration (in mol dm^{-3}/55.5), the mean aggregation number M of elastic bilayer vesicles will be given by Eq. (16.23) as

$$M = \sqrt{Ce^{\alpha}} = e^{\pi k_b/kT}\sqrt{C} \qquad (17.21)$$

and the number density of vesicles will decay exponentially with their aggregation number, N, according to Eq. (16.25):

$$\text{vesicle distribution} \quad X_N/N = \text{Const. } e^{-N/M}. \qquad (17.22)$$

Thus, for typical dilute lipid concentrations of $C = 10^{-4}$ mol dm^{-3} and below, we see that if $k_b > 2 \times 10^{-20}$ J, vesicles will be large ($M > 10\,000$) and polydisperse, and their mean size will increase with the total lipid concentration (according to $R \propto C^{1/4}$). But if $k_b < 2 \times 10^{-20}$ J, the bending modulus is too low to have any significant effect, and vesicle populations will now be small and monodisperse, with a mean radius $R \approx R_c$ determined by the critical packing parameter (Fig. 17.5).

Eventually, at high enough concentrations, large vesicles must always transform into extended bilayers, either because of space requirements or because of the attractive forces between them, as discussed in Section 16.8.

Negative curvature modulus ($k_b < 0$)

A negative elasticity favours bending of a bilayer right from the start and leads to smaller vesicles than expected from simple packing considerations. The effect is enhanced for large repulsive headgroups, as well as for shorter hydrocarbon chains. Thus, below a certain chain length no stable vesicles should form; instead cylindrical or spherical micelles become the preferred structures as occurs for double-chained lecithins with 12 or fewer carbons per chain.

The above analysis should apply to any spherical structure in solution bounded by an elastic bilayer, monolayer or membrane, for example, microemulsion droplets. Figure 17.5 shows how the molecular interaction energy and size distribution of a vesicle population depends on the relative magnitudes of the bending modulus and molecular packing.

The simple 'packing model' developed so far takes us about as far as one

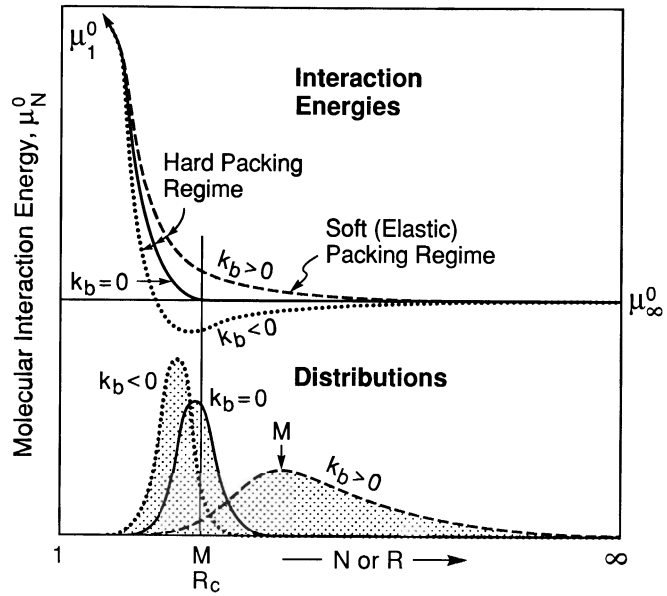


Fig. 17.5. Schematic variation of interaction energy per molecule in a vesicle with aggregation number N. Solid lines: no curvature elasticity ($k_b = 0$). Dashed lines: positive curvature elasticity ($k_b > 0$). Dotted lines: negative curvature elasticity ($k_b < 0$). The corresponding distributions of vesicle sizes (X_N/N) is also shown. Note that for large positive k_b the distribution is determined mainly by k_b (soft packing regime), while for small or negative k_b it is determined by $v/a_0 l_c$ (hard packing regime).

can go without resorting to much more sophisticated theoretical methods in which the various intermolecular and interaggregate interactions are treated to a full statistical thermodynamic analysis or a computer simulation. These have recently been extended from looking at small micelles (Jönsson and Wennerström, 1981; Gruen, 1985; Szleifer *et al.*, 1985, 1986; Jokela *et al.*, 1987; Blanckstein *et al.*, 1986) to looking at bilayers (Leermakers and Scheutjens, 1988; Egberts and Berendsen, 1988; Cevc and Marsh, 1987; De Loof *et al.*, 1991) including interbilayer interactions (Granfeldt and Miklavic, 1991) and even more complex self-assembling structures and biological membranes (Pastor, 1990).

17.10 BIOLOGICAL MEMBRANES

Membranes are the most common cellular structures in both animals and plants (Fig. 17.6) where they are involved in almost all aspects of cellular

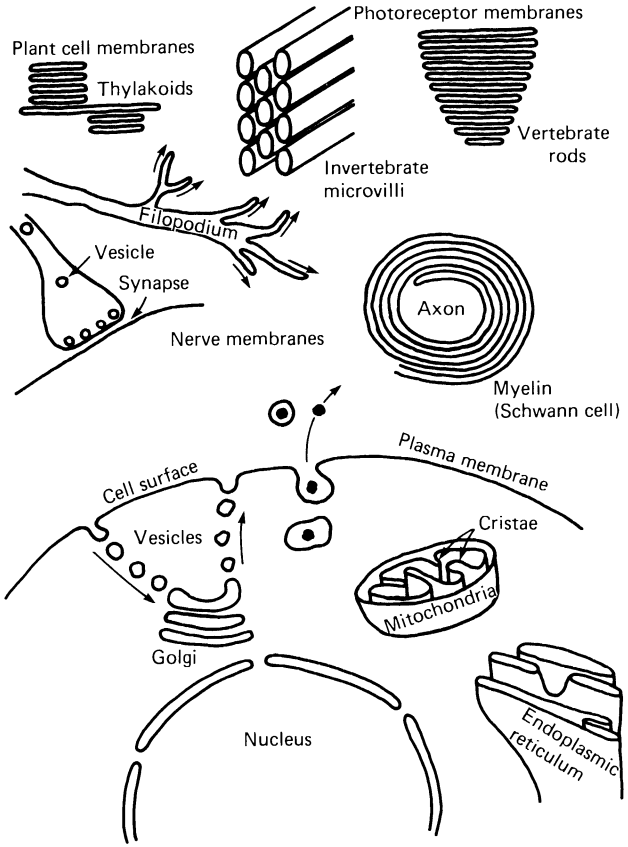


Fig. 17.6. Cellular membranes are thin sheets of lipids and proteins. Most biological membranes offer little resistance to bending.

activity ranging from simple mechanical functions such as motility, food entrapment and transport to highly specific biochemical processes such as energy transduction, immunological recognition, nerve conduction and biosynthesis (for general books on biomembranes see Bittar, 1980; Harrison and Lunt, 1980; Jain, 1988). Biological membranes are very complex and varied. They commonly contain 50 or more different proteins and a host of phospholipids and glycolipids with various headgroups, numbers of chains, chain lengths and degrees of unsaturation, as well as steroids (e.g., cholesterol) and other amphiphilic molecules. Yet in spite of their complexity there are many aspects of membrane structure that may be qualitatively understood in terms of the concepts we have already outlined, and it is best to start with a consideration of membrane lipids.

17.11 MEMBRANE LIPIDS

Most biological membrane lipids are *double-chained* phospholipids or glycolipids, with *16 to 18* carbons per chain, one of which is *unsaturated* or *branched* (see Table 16.1). These properties are not accidental but carefully designed by nature to ensure

(i) that biological lipids will self-assemble into thin bilayer membranes that can compartmentalize different regions within a cell as well as protect the inside of the cell from the outside;

(ii) that because of their extremely low CMC the membranes remain intact even when the bathing medium is grossly depleted of lipids; and

(iii) that because of the unsaturation or branching the membranes are in the fluid state at physiological temperatures.

For example, as shown in Table 17.1, the chain melting temperatures T_c of saturated di-C_{18} phospholipids are well above room temperature, whereas the unsaturated lipids have their T_c below 0°C. As a consequence of this fluidity most biological membranes can easily deform and bend until limited by packing constraints. Fluid membranes also allow various solute molecules to pass through them and protein molecules to diffuse along them.

A further important aspect of lipid chain fluidity is that different lipid types can pack together, that is, mutually accommodate each other as well as other molecules, while remaining within a planar or curved bilayer configuration. In addition, the curvature can be regulated by altering the ratio of its constituent lipids. For example, addition of cone-shaped lipids such as lysolecithin (Table 17.2) to lecithin vesicles results in smaller vesicles since a mixture of such lipids can pack into more highly curved bilayers. Actually, only small amounts of single-chained lysolipids can be incorporated into bilayers or biological membranes before they break up (become solubilized) into small vesicles or micelles.

On the other hand, addition of wedge-shaped lipids such as phosphatidylethanolamine and cholesterol increases the radius of bilayers, straightens the hydrocarbon chains and in general reduces the fluidity (thereby causing the 'hardening') of membranes. Again, only a limited amount of cholesterol and phosphatidylethanolamine can be incorporated into bilayers before the bilayer structure is destroyed, at the point where the fluid lipids can no longer accommodate these wedge-shaped lipids. It is probably for this reason that natural biological membranes never contain high amounts of both cholesterol and phosphatidylethanolamine (the two most common wedge-shaped lipids in animal membranes). As might be expected, cholesterol and lysolecithin—neither of which can form a stable bilayer by itself—can combine in certain