MICELLAR SOLUTIONS AND LIPID VESICLES

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Abstract. - Amphiphilic molecules containing a strongly polar head group attached to one or more hydrocarbon chains spontaneously form organized structures in an aqueous medium in which contact between hydrocarbon and water is minimized. Phospholipids are the most important biological molecules of this kind. They have two hydrocarbon chains per head group and their thermodynamically preferred state is a vesicle, bounded by a single bilayer with a central hydrocarbon core, and enclosing an aqueous cavity. Molecules with single hydrocarbon chains form small disk-shaped micelles with a central hydrocarbon core. The hydrocarbon micro-phases in both systems are liquid, but molecular motion is restricted by geometrical constraints. Nevertheless both systems have solvent properties similar to those of bulk hydrocarbon and are thus capable of forming discrete pockets of nonaqueous solution segregated from the enveloping aqueous medium.

INTRODUCTION

It seems paradoxical that someone like myself, a biological scientist exclusively preoccupied with aqueous media, should be invited to present a lecture at an international conference on nonaqueous solutions. However, we have islands of hydrocarbon in the midst of our oceans of water, and life could not exist without them. These islands of hydrocarbon are the subject of this paper. They are formed by amphiphilic molecules, molecules that are strongly hydrophilic at one end and strongly hydrophobic at the other end. The most important molecules of this kind in living systems are phospholipids, which contain two long hydrocarbon chains attached to a charged phosphoglyceride head group. The same chemical principles apply, however, to the molecules of soaps and detergents, which contain only a single hydrocarbon chain, attached to any of a large variety of charged or polar head groups. I shall discuss both systems in this paper. In both systems the presence of the opposing hydrophobic and hydrophilic driving forces on the same molecule leads to molecular organization into distinct chemical domains, and these domains have the unique property of being in a liquid state. I have discussed the basic physical chemical principles governing these systems in my book The Hydrophobic Effect (Ref. 1) and have described their critical importance to the existence of living cells in an earlier article (Ref. 2).

MONOLAYERS, MICELLES AND VESICLES

Benjamin Franklin gave the first scientific description of the molecular organization produced by amphiphilic molecules in a remarkable paper published in 1774 (Ref. 3). Franklin wanted to test the well-known principle that oil can be used to reduce the height of waves on a stormy sea. The word "oil"

in those days did not refer to paraffin oil, as it does now, but to vegetable oil, which consists of amphiphilic molecules closely related to phospholipids, but usually containing three instead of two hydrocarbon chains per molecule. On a windy day in London, he dropped a teaspoon of oil on the surface of Clapham Pond, which had a rough surface because of the wind, and he indeed observed that the oil made the surface very smooth. But Franklin observed something else: the smoothing effect spread out over a very large part of the pond, to an area which Franklin estimated as $2000m^2$. This means that the oil contained in the small volume of a teaspoon must have become spread out over the same area, and Franklin's keen scientific intuition made him realize that this observation was at least as important as the smoothing of the waves itself. He tried seriously to explain it in molecular terms, and recognized that a lack of attraction between water and oil molecules had to be one factor, because otherwise the oil would have dissolved in the water. But he also recognized that this could not be a sufficient explanation and struggled hard to try to define the second force that must be involved. Had he been able to imagine the possibility that a single molecule could have different attributes at opposite ends of the molecule, he would almost certainly have realized that explanation of the spreading phenomenon requires both attraction and repulsion. In 1774 this idea was inconceivable. It was accepted that each chemical substance is made up of characteristic ultimate particles, which we now call molecules, but it was assumed that each molecule would possess the same attributes as the bulk substance throughout its mass. The possibility that one end of a vegetable oil molecule can be attracted to water, and the other end repelled by it, could not have been conceived. We know today, of course, that the area of $2000m^2$ in fact corresponds to the area of a closely packed unimolecular film in which each individual molecule satisfies the dual thermodynamic drives of its amphiphilic nature by having its hydrophilic part in the water, and its hydrophobic part sticking out of the water. The close packing assures that the hydrocarbon chains are still in close contact, as required by the van der Waals attraction between them.

Most laboratory experiments with amphiphilic molecules are conducted under conditions where the available surface is extremely small and the same is true in living cells where lipids are synthesized. Under these conditions amphiphilic molecules have to create their own nonaqueous environment and phospholipids do so by forming an extended bilayer, a bimolecular sheet in which hydrocarbon chains form an internal layer (thickness about 3nm) which is sandwiched between two external layers created by the polar lipid head groups (Fig. 1). Contacts between hydrocarbon and water are therefore virtually eliminated, except at the outer edge of the bilayer. In moderately dilute solutions, where lipid is likely to form many small separate patches of bilayer, these residual edge contacts still constitute a source of thermodynamic instability, and the bilayer patches will then spontaneously adopt a vesicular form, whereby edge contacts are completely eliminated. It should be emphasised that no special skills are required to form such vesicles. The process is completely spontaneous, requiring only that the lipid molecules are initially dispersed with sufficient molecular mobility to give them a chance to rearrange themselves into their thermodynamically most stable state.

It is possible to prepare vesicles with functionally intact proteins from cell membranes inserted into the vesicle wall (Ref. 4 & 5). Such protein containing vesicles can be considered to be prototypes of living cells for many purposes. The simplest living cells are in fact nothing more than concentrated solutions of DNA, enzymes, metabolites, etc, sealed off from their environments by a single phospholipid bilayer membrane. The living membrane contains many different kinds of protein molecules, which provide regulated means of communication between inside and outside. By purifying these proteins, and inserting them into artificial vesicles in the purified state, we are able to study the functional properties of individual membrane proteins separately, and this makes phospholipid vesicles powerful tools for biochemical and physiological research. This is basically the reason why we are interested in these vesicles in my laboratory. We are chiefly interested in the proteins that permit regulated transport of ions between the two aqueous phases shown in the diagram of Fig. 1. We are less concerned with the intervening narrow layer of liquid hydrocarbon, which serves as a permeability

HYDROCARBON   HEAD GROUP
(30 Å)   LAYERS

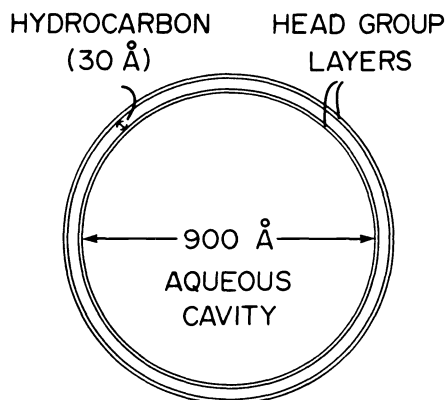900 Å
AQUEOUS
CAVITY

Fig. 1. Cross-sectional representation of a spherical
phospholipid vesicle. A vesicle with a diameter of 100
nm (1000 Å) is bounded by a bilayer containing about
85,000 lipid molecules (Ref. 5). Vesicles are readily
prepared with diameters ranging from 25 to 250 nm.

barrier between the aqueous phases. Our interest in this phase is limited
to properties that might affect the proper functioning of the inserted pro-
teins. One essential factor is that the hydrocarbon phase must be liquid,
so that it can form a proper seal around the inserted proteins, and for this
reason we always use phospholipid molecules that contain a mixture of unsa-
turated and saturated hydrocarbon chains, simulating what nature does in
living membranes. If lipids with identical long saturated hydrocarbon
chains are used, the hydrocarbon tends to adopt an ordered crystalline ar-
rangement.

When single chain amphiphiles are placed into an aqueous medium, they are
subject to the same thermodynamic forces as two-chain amphiphiles, and
therefore have the same tendency to adopt a bilayer arrangement.   Single
chain amphiphiles, however, contain twice as many head groups per hydrocar-
bon chain and the head groups would therefore be very closely packed in an
extended bilayer sheet. Such close packing is thermodynamically unfavorable
under most circumstances, and a modification of the bilayer structure to
achieve a curved surface and a greater separation between head groups be-
comes necessary. As a result, single chain amphiphiles normally form small
disk-like micelles containing of the order of 100 amphiphile molecules per
particle (Ref. 1 & 2). The necessary surface curvature induces a gradual
change in orientation of the hydrocarbon chains as one moves from the center
of the disk towards its edge. The result is a small droplet of hydrocarbon
surrounded by a surface layer of polar head groups. The asymmetry of the
disk (ratio of diameter to height) is usually not large, but the ultimate
symmetry of a spherical particle is not attained because the length of the
hydrocarbon chain limits the radius, and therefore also the volume, that a
sphere could have. As a result, spherical micelles necessarily have a small
aggregation number, which leaves a large area of hydrocarbon/water contact
between head groups and leads to a residual thermodynamic force in favor of

a decrease in the average surface curvature. A disk-like micelle is the appropriate compromise between the too large separation between head groups in a sphere and the excessively tight packing in an extended bilayer.

I should emphasize that vesicles and micelles are the preferred structures in these systems only in dilute solutions. Various kinds of lipid-rich and detergent-rich phases can be formed in concentrated solutions with a low water content. Phospholipids under these conditions form multi-layered assemblies consisting of many bilayers lying on top of one another, with relatively thin aqueous layers between the bilayers. This arrangement is suitable for crude x-ray diffraction, and much useful information about bilayers has been obtained in this way (Ref. 6). The results are directly applicable to bilayers in the vesicular state or in cell membranes because the individual bilayers in a multi-layered arrangement are not altered significantly by being packed close to each other. The same statement cannot be made for ordered structures that are formed by detergents at low water content. Formation of these ordered structures involves complete rearrangement of the amphiphile molecules (Ref. 7), so that no information about soluble micelles can be obtained from x-ray diffraction studies.

PROPERTIES OF THE HYDROCARBON PHASE

For the biochemist or physiologist, phospholipid vesicles are of course much more interesting than small micelles because of their sealed-off aqueous space. In relation to the chemistry of nonaqueous solutions, however, the hydrocarbon phase in these systems is presumably the subject of greatest interest. From this point of view there is little difference between vesicles and micelles. At a superficial level the hydrocarbon phases of both systems can be regarded as resembling bulk liquid hydrocarbon. For example, amphiphiles with reasonably compatible head groups are freely miscible, and we can mix single chain and double chain amphiphiles, forming enlarged micelles when the single chain amphiphile is in excess and vesicles with an increased tendency to rupture when the double chain amphiphile is in excess. As another example, the permeability properties of bilayers are approximately those that we would expect on the basis that solubility of the permeant in the hydrocarbon phase is the determining factor (Ref. 8 & 9). The walls of phospholipid vesicles are thus highly impermeable to small ions (Ref. 5) and to large polar molecules, but highly permeable to alcohols and to nonpolar organic solutes. They also have a relatively high permeability for water (Ref. 9), which, presumably because of its small molecular size, has a fairly high solubility in liquid hydrocarbons as well.

This view of the micellar or vesicular hydrocarbon phase is of course only a crude approximation. The configurations of the hydrocarbon chains in these systems have to be severely constrained by the continuity of the chains and by the fact that one end of each chain is anchored to a polar head group at the aqueous interface. This requires that adjacent hydrocarbon chains have to be more or less parallel to each other and perpendicular to the interfacial surface. Three dimensional tumbling of the chains is absolutely prohibited. The fluidity of this particular kind of liquid is thus limited largely to molecular flexibility of individual chains, and intertwinning of chains, if it occurs at all, is confined to the chain ends near the center of the micelle or bilayer. The consequences of these constraints for phospholipid bilayers have been investigated very thoroughly, both theoretically and experimentally. When the hydrocarbon chains of the phospholipid are saturated, ordered crystalline phases are readily formed, and the melting points of these ordered structures are much higher than in bulk hydrocarbon. For example, dipalmitoyl phosphatidylcholine (pentadecyl hydrocarbon chain) has a melting point of 41°C whereas crystalline pentadecane itself melts at 10°C (Ref. 6). The reason for this is the restricted motional freedom of the hydrocarbon chains in the fluid state of the bilayer, as a result of which the entropy gained on melting is less than for the pure alkane. Detailed studies by use of nmr (Ref. 10) show that there is a gradient in motional freedom across the bilayer: CH_2 groups closest to the interface are in a highly ordered state and disorder increases progressively as one approaches the center of the bilayer.

As I mentioned earlier, we ourselves are mostly interested in bilayers con-

taining unsaturated hydrocarbon chains. Here there is no tendency to form crystalline phases, and the fluid hydrocarbon phase is more disordered than in bilayers with saturated hydrocarbons above their melting points. Nevertheless, the chains still cannot tumble and must still be more or less parallel to each other, especially near the bilayer surface. The unsaturated hydrocarbon chain that occurs most commonly in lipids used for experimental studies is derived from oleic acid, which has its double bond in the middle of the hydrocarbon chain and therefore about halfway between the surface and the center in a bilayer arrangement. The central part of the bilayer still constitutes the most disordered region, and small nonpolar solutes dissolved in a bilayer are likely to reside in the central part rather than near the surface.

Single chain amphiphiles used for experimental studies of micellar solutions usually have saturated alkyl chains, somewhat shorter in length than hydrocarbon chains of phospholipids. Dodecyl chains are typical. Both because of the shorter chain length and the curved surface, crystallization of the hydrocarbon chains in the micellar state does not occur. Recent theoretical work by Dill and Flory (Ref. 11) suggests that freedom of motion of the hydrocarbon chains in micelles should be greatest near the external surface because, as a result of the surface curvature, the available space becomes progressively more restricted as the center of the micelle is approached. These geometrical considerations suggest that non-polar solutes dissolved in micelles might be likely to reside near the micellar surface, in contrast to the opposite situation in bilayers (Ref. 11). This may be true for alcohols, halobenzenes and other molecules that have weakly polar groups that can intercalate between amphiphile head groups, but it cannot be true for totally nonpolar solutes because the hydrophilic nature of the micelle surface has to be preserved. In other words, totally nonpolar solutes can be dissolved only to the extent that one can increase the volume of the micellar hydrocarbon phase without significantly increasing the surface area. For disk-like micelles this is actually not a serious limitation provided that the dissolved solute resides at the center of the micelle. In that position it removes the restriction on the diameter that a spherical micelle can possess, which is now no longer limited to the length of two amphiphile hydrocarbon chains. An increase in volume can therefore be compensated by a change in shape from disk-like towards spherical, so that the surface area can remain roughly constant. These geometrical considerations suggest that micellar and bilayer hydrocarbon phases should behave quite similarly with respect to solubilization of nonpolar solutes, but both should differ from bulk hydrocarbon because of the restricted motional freedom in the micelle or bilayer interior. The limited experimental studies that have been done support this conclusion.

TABLE 1. ΔG^{o} for transfer from aqueous to organic phase in kJ/mol at 25°C.

Solvent phase	Solute	
	Hexane	Benzene
Benzene		-19.7
Hexane	-32.4	-18.0
Na dodecyl sulfate micelles	-27.4	-17.4
K dodecanoate micelles		-18.0
Dioleoyl lecithin bilayers	-24.1	-18.2
Egg lecithin bilayers	-26.1	-18.2

ΔG^{o} is for mol fraction standard state. For lipid bilayers each acyl chain is regarded as independent in calculating mol fractions. Data from Refs. 12 & 13.

Table 1, for example, shows standard free energies of transfer of benzene and hexane between aqueous solutions and hydrocarbon phases, measured at fairly low concentrations, so that they can be taken as an approximate representation of infinite dilution values. (The choice of an aqueous solu-

tion as a reference state reflects the prejudice of a biological scientist. The data could equally well have been given in terms of pure hexane or ben- zene as reference state.) The data shows that the organizational restric- tions within micelles and bilayers have little effect on the thermodynamic state of benzene, presumably because benzene is a small and rigid molecule (Ref. 12). Benzene is at a slightly higher chemical potential in bulk liquid hexane than in an aromatic solvent, perhaps because the rigidity of the aromatic ring slightly restricts the freedom of motion of adjacent hex- ane molecules. The more important result is that there is no additional thermodynamic effect in going from liquid hexane to the interior of a mi- celle or bilayer. When hexane is used as solute, however, there is a larger difference between liquid hydrocarbon and a micellar solution, and this is undoubtedly due to the fact that the hexane molecule is flexible and has considerable freedom of intramolecular motion in bulk hydrocarbon. This freedom would be restricted in a micellar solution in the same way as the freedom of motion of the amphiphile hydrocarbon chains themselves. There is an additional small difference in free energy between the micelle interior and a phospholipid bilayer, which is not surprising if loss of motional freedom is the determining factor (Ref. 13).

It is probable that the effects of restricted freedom of motion on solubil- ized alkanes will become more severe as the alkane chain length is in- creased. There are no dilute solution studies from which data analogous to those of Table 1 can be derived, but there is a dramatic effect of chain length on total solubility to the extent that alkane molecules that are longer than the hydrocarbon chains of the amphiphile molecules of the solu- bilizing particle become virtually insoluble. Measurements of alkane solu- bility in micelles that demonstrate this effect were reported more than 30 years ago (Ref 14). Fig. 2 shows similar data for phospholipid bilayers (Ref. 15). These are not direct solubility data, but show instead the thickness of the hydrocarbon region as measured by x-ray diffraction studies of multi-layer assemblies which had reached saturation with respect to the amount of hydrocarbon they could accommodate. The bilayer thickness can be taken as a valid measure of the increase in the volume of the hydrocarbon region because the surface area cannot be altered significantly when the number of polar head groups in the bilayer remains the same. The results show that the bilayer thickness at saturation decreases steeply with in- creasing alkane size: for hexadecane the thickness is essentially the same as for the unperturbed bilayer in the absence of added alkane.

Another aspect of the data of Fig. 2 is that they demonstrate that the high- ly soluble smaller alkanes must be mostly located at the bilayer center, in
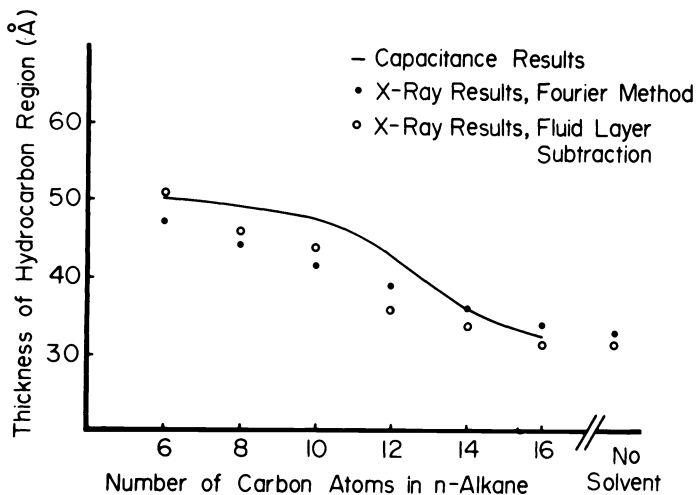

Fig. 2. Thickness of the hydrocarbon layer in a lipid bilayer saturated with dissolved alkanes of different chain length. The two sets of points represent alterna- tive ways of estimating hydrocarbon thickness from x-ray diffraction measurements of ordered multi-layered assem- blies of egg phosphatidylcholine (Ref. 15). The solid line is based on measurements of electrical capacitance for a single planar bilayer (Ref. 6). Figure provided through the courtesy of Dr. T.J. McIntosh.

the space between the chain ends of the phospholipid hydrocarbon chains.
Bilayers can to some extent absorb additional hydrocarbon by intercalation
between the lipid hydrocarbon chains. The latter would be straightened out
and lengthened in the process, and could approach the fully extended length
of an all-trans configuration. The observed increase in bilayer thickness
for the smaller alkanes is much greater than can be accounted for in this
way (Ref. 15).

KINETICS OF VESICULATION

One of the notable features of the organization of amphiphilic molecules is
that it is a thermodynamic phenomenon. Micelles and vesicles represent true
equilibrium states resulting from the quest of the constituent molecules for
their individual states of lowest chemical potential. One aspect that is
however not entirely thermodynamic is the size of phospholipid vesicles,
which, as shown in Table 2, depends on the method used for vesicle prepara-
tion. One way to achieve dispersal of phospholipid for subsequent vesicula-
tion is to employ sonication (Ref. 17). Vesicles that are formed when the

TABLE 2. Size of phospholipid vesicles.

Preparative method	Outside diameter,nm	Ref.
Sonication	25	(17)
Detergent solubilization and removal		
using cholate	30	(18)
using $C_{12}E_8$	130	-
using octyl glucoside	240	(5)
Ether injection and evaporation	250	(20)

Vesicle preparations are moderately heterogeneous, and
listed diameters are approximate average values. $C_{12}E_8$
is dodecyl octaethyleneglycol monoether. Size estimate
for this preparation is an unpublished result from this
laboratory.

sonicator is turned off are invariably very small, with an external diameter
of less than 30nm and an extremely small internal aqueous cavity. Another
way to achieve dispersal is to dissolve the phospholipid in an excess of
detergent, forming mixed micelles. The detergent can be removed by a
variety of techniques, and spontaneous vesicle formation occurs (Ref. 5 &
18). Different vesicle diameters are obtained, depending on the detergent
employed. An alternative procedure is to dissolve lipid by adding an organ-
ic solvent to an aqueous lipid suspension and then to remove the organic
solvent by evaporation. Diethyl ether is the principal organic solvent em-
ployed for this purpose so far, and the vesicles formed by its use are among
the largest that have been reported (Ref. 19 & 20). An important aspect of
these data is that the vesicles retain their characteristic size for many
weeks, regardless of which method is used. Once the bilayer edges have
closed upon themselves to create the vesicular state, exchange of consti-
tuent molecules between vesicles, or other processes that might affect vesi-
cle size distribution, become virtually impossible because the phospholipid
molecules are in a thermodynamic sink from which they cannot escape.

There has been no systematic study of the kinetics of vesicle formation that
could provide a theoretical explanation for the observed size differences.
Bilayers presumably become locked into vesicular form before all detergent
or organic solvent has been removed, and the amount of residual detergent at
the time of vesiculation will certainly affect the bilayer curvature. Since
vesiculation itself is a slow process, occurring on a time scale of minutes,
it seems likely that the rate at which detergent is removed from the mixture
may affect vesicle size, and preliminary experiments support this (J.A. Rey-
nolds, personal communication). The results must however also be influ-
enced by specific geometrical factors unique to each detergent: the surface
curvature at a given detergent level can be expected to be influenced by the
precise mode of interaction between detergent and lipid head groups.

INTERFACIAL TENSION AND LAPLACE PRESSURE

The equation of Laplace for pressure differences across curved interfaces has been used to suggest that both micelles and lipid vesicles have high internal hydrostatic pressures (Ref. 21 & 22). This conclusion is erroneous (Ref. 23), as the following derivation demonstrates. The derivation may be of general interest, quite apart from the question of internal pressure, because it provides a particularly useful way of perceiving the physical basis for the formation of micelles and bilayers in terms of the opposing thermodynamic tendencies within amphiphile molecules.

If an ionic or highly polar solute is placed into an aqueous medium, there are attractive forces between solute and solvent and the free energy of the system is decreased by increasing the solute/solvent interfacial area. Maximization of the interfacial area is thermodynamically favored and this is achieved by complete solution of the solute, as illustrated by curve 1 in Fig. 3. When pure hydrocarbon is placed into water, on the other hand, solute/solvent contacts are thermodynamically unfavorable. The excess free energy attributable to such contacts, for aliphatic hydrocarbon molecules, is close to a linear function of contact area, about 100 J mol^{-1} per Å^2 of contact area for a dissolved hydrocarbon particle (Ref. 24). If we can prevent the hydrocarbon from escaping from the aqueous phase, its minimal free energy state will be the smallest possible spherical condensed globule (curve 2 of Fig. 3). In this case the law of Laplace applies. The interfacial area can shrink to a slightly smaller value than otherwise if the hydrostatic pressure within the sphere is increased. The law of Laplace (Ref. 25) defines the equilibrium pressure difference (ΔP) across the interface in terms of the interfacial tension γ as

$$\Delta P = 2\gamma/R \qquad (1)$$

where R is the radius of the sphere. The interfacial tension γ can be defined in terms of the slope of curve 2,

$$\gamma = (\partial F/\partial A)_{T,V} \qquad (2)$$

and is of course positive for all values of the area A because of the linear relation between free energy and area.

The situation for an amphiphile is quite different. Complete dispersal in the solution is thermodynamically unfavorable because of the hydrocarbon/water contacts in that condition, i.e., at high interfacial areas the amphiphile behaves like a hydrocarbon. However, compression into
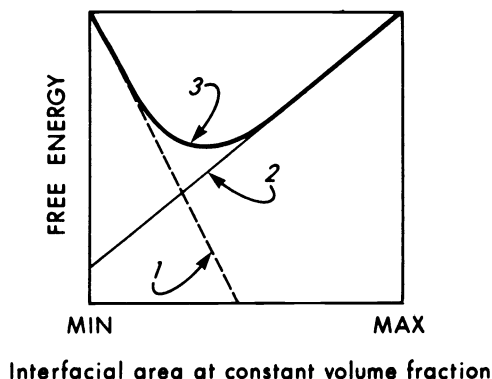

Interfacial area at constant volume fraction

Fig. 3. Effect of changing interfacial contact area on free energy in an aqueous medium containing a fixed volume fraction of solute. The maximal area corresponds to complete solution of the solute as individual molecules. The minimal area corresponds to solute packed into a single spherical droplet. Curve 1 is for an ionic or highly polar solute, free energy becoming very negative for complete dispersal. Curve 2 is for an inherently immiscible additive, such as pure hydrocarbon. Curve 3 is for an amphiphilic solute.

a small sphere is also thermodynamically unfavorable, because the hydrophilic end of the amphiphile is then not satisfied, as Franklin's spreading experiment dramatically illustrates. Amphiphiles therefore behave like ionic or polar solutes at small values of the interfacial area, with $\partial F/\partial A$ formally negative instead of positive. As is illustrated by curve 3 of Fig. 3, the state of lowest free energy for an amphiphile is attained at an intermediate value for the surface area. From the point of view of surface chemistry, we can thus regard both micelles and vesicles as representing aggregation states of minimal surface free energy. At this thermodynamic minimum, $\partial F/\partial A = 0$, and this indicates that micelles and vesicles represent states of zero interfacial tension. As a consequence, by equation 1, the Laplace pressure within micelles and vesicles must also be essentially zero.

POINTS OF CONTROVERSY

Hydrodynamic measurements often indicate the presence of large rod-shaped particles in amphiphile solutions, especially at high concentrations (Ref. 26 & 27). In most cases these data are interpreted at face value and are used to suggest that micelles commonly have a rod-like shape (Ref. 11), contrary to my previous assertion that micelles generally have a disk-like shape. The rod-shaped particles are in fact obtained under conditions where secondary association between micelles is likely to occur. Ionic micelles at high ionic strength represent one example (Ref. 28): counterions at high concentration can create weak bonds that could cause micelles to become self-associated when the micelle concentration is large. If the primary micelles are disk-like, the aggregation process would be expected to occur between the broad faces of the disks, leading to rod-like particles.

An example of perhaps greater interest for this symposium is provided by the nonionic micelles formed by alkyl ethers of polyoxyethylenes, in which the polar group of the amphiphile molecule is a short polyoxyethylene chain of variable length, up to about 30 oxyethylene units. Micelles formed by these amphiphiles have head group regions that extend far out from the central hydrocarbon core (Ref. 29). The head group regions have uniform thickness, so that the primary micelles have a shape that is difficult to distinguish from a spherical shape even if the hydrocarbon core of the micelle is itself disk-like. These micelles can form extremely large rod-like particles at high amphiphile concentrations if the polyoxyethylene head group is short (Ref. 1 & 26). The formation of large particles is characterized by a positive enthalpy, whereas primary micelle formation is normally nearly independent of temperature. In addition, measurements of relaxation times by nmr (Ref. 30) indicate that the large particles contain within them smaller particles that are capable of independent motion. The evidence favoring secondary association is therefore in this case persuasive. The forces involved may be similar to the forces that lead to phase separation in aqueous solutions of pure polyoxyethylenes. The phenomenon merits detailed study: the effects of polar group chain length and of temperature are very striking and their quantitative theoretical explanation may be able to make a considerable contribution to our understanding of molecular organization in liquid mixtures.

A second area of controversy is the frequent assertion, based on nmr measurements, that water molecules penetrate deep into the interior of small micelles (Ref. 31). On thermodynamic grounds a large equilibrium solubility of water in the micelle interior seems improbable, and it has been pointed out (Ref. 32) that the nmr data used to infer water penetration are susceptible to alternative interpretations. In the case of bilayers, the virtual absence of water from the hydrocarbon phase has been demonstrated by neutron diffraction measurements. These measurements have been able to show that the large effects resulting from substitution of D_2O for H_2O in multibilayer assemblies are confined to the region between bilayers (Ref. 33).

CONCLUSIONS

One of my objectives in this paper has been to stress the similarity between the hydrocarbon micro-phases of spread amphiphile monolayers, micellar solutions, phospholipid vesicles, and phospholipid bilayers in multi-layered assemblies. The hydrocarbon regions in these systems are more ordered than bulk liquid hydrocarbon, but they retain the thermodynamic properties of bulk liquid hydrocarbon. In particular, they are all capable of dissolving a large variety of small organic solutes, and thus able to create discrete regions of nonaqueous solution which are unique in having microscopic dimen-

sions of the order of the size of individual molecules. They therefore pro-
vide a means for studying intermolecular interactions at a more intimate
level than is possible in bulk solution, under conditions where the relative
geometrical orientation of the interacting molecules is limited and easily
visualized. I have illustrated the potential value of this kind of arrange-
ment by use of equilibrium distribution data, but nmr and other spectroscop-
ic methods can of course potentially be even more powerful tools for this
type of application (Ref. 34 & 35).

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