The Hydrophobic Effect and the Organization of Living Matter

Charles Tanford

One of the principal objects of theoretical research in any department of knowledge is to find the point of view from which the subject appears in its greatest simplicity. —J. WILLIARD GIBBS, in a letter to the American Academy of Arts and Sciences, January 1881

Summary. Biological organization may be viewed as consisting of two stages: biosynthesis and assembly. The assembly process is largely under thermodynamic control; that is, as a first approximation it represents a search by each structural molecule for its state of lowest chemical potential. The hydrophobic effect is a unique organizing force, based on repulsion by the solvent instead of attractive forces at the site of organization. It is responsible for assembly of membranes of cells and intracellular compartments, and the absence of strong attractive forces makes the membranes fluid and deformable. The spontaneous folding of proteins, however, involves directed polar bonds, leading to more rigid structures. Intercellular organization probably involves polar bonds between cell surface proteins.

It is almost exactly 100 years since the publication of Gibbs' paper (*1*) "On the equilibrium of heterogeneous substances." The time now seems appropriate to use his method of thermodynamic analysis as a basis for thinking about the organization of the living cell and, beyond that, the assembly of cells into multicellular organisms. One should not be under the illusion that such an analysis will produce dramatic new advances, such as might result from the discovery of a hitherto unknown chemical substance or from the determination of the three-dimensional structure of an important biological macromolecule. But science does not progress by startling discoveries alone, and thermodynamic analysis serves its purpose by assimilating the new into the old and the part into the whole. It can create a simple unified conceptual framework for biology, as it has done for chemistry, and has the potential for clarifying research problems and indicating productive pathways for solving them.

It might at first seem an unrewarding task to apply equilibrium thermodynamics to biology at all, for life is surely the antithesis of an equilibrium state. In fact, however, the dynamic processes of life take place within an organized structural framework that turns over slowly or not at all. It is realistic to consider this framework (tentatively, as long as no compelling contrary evidence presents itself) as being essentially at equilibrium, subject only to spatial and temporal constraints that are usually self-evident. Such constraints, in the form of physical or kinetic barriers, exist whenever the laws of equilibrium thermodynamics are applied, and the constraints in living systems are no different from those encountered in simple chemical systems.

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Thermodynamic Background

The great achievement of Gibbs was the definition of the chemical potential (*2*), which permits the state of equilibrium in a complex chemical system to be defined precisely, and often as simply as in a purely mechanical or electrical system. In this article we are interested in the assembly of molecules into organized structures, without chemical interconversions—that is, in the distribution of molecules between various phases or places in the biological system. The Gibbs equilibrium condition is that the chemical potential (μ_i) of each definable component must have the same value in each phase or place accessible to it; that is

$$\mu_{ia} = \mu_{ib} = \mu_{ic} = \ldots \quad (1)$$

where the subscripts a, b, c, . . . designate the possible locations of each component.

The phases with which we deal are solutions or mixtures, and the free energy includes a major contribution from the statistical entropy of mixing, which is nonspecific and does not depend on what substances are being mixed. Most workers have followed the procedure of Gurney (*3*), segregating this nonspecific statistical factor from the more interesting specific parts of the chemical potential. The procedure is based on an ideal expression for the entropy of mixing, and leads to $RT \ln X_i$ (where R is the gas constant, T is absolute temperature, and X_i represents mole fraction) as the purely statistical contribution to each μ_i. Equation 1, for any two environments, can then be rewritten as

$$\mu^0_{ia} + RT \ln X_{ia} = \mu^0_{ib} + RT \ln X_{ib} \quad (2)$$

where μ^0_i is the specific part of the chemical potential, called by Gurney the unitary potential. It includes the free energy of the inherent molecular motions of an isolated molecule of type i, plus the free energy arising from specific interactions with neighboring molecules, which are solvent molecules in a dilute solution or adjacent molecules of type i in a pure phase. Both factors are the sums of the enthalpy and entropy contributions; that is, $RT \ln X_i$ is not to be thought of as representing the total contribution of entropy to the chemical potential.

The thermodynamics of biological organization does not involve biosynthesis or other chemical transformations, but focuses solely on where molecules prefer to go after they have been synthesized. The properties of the isolated molecules on the two sides of Eq. 2 are the same, and the difference between μ^0_{ia} and μ^0_{ib} therefore comes exclusively from interactions with adjacent solvent or like molecules. It is moreover implicit in the use of the term "organization" that the distributions of interest are lopsided, greatly favoring one environment over another, so that one is always concerned with large differences in the unitary potential. Because of this, the possible error arising from use of an ideal expression for the entropy of mixing, which could in principle lead to incorpo-

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ration of part of the true mixing entropy into μ^{o}_{i}, is probably unimportant (*4*). I conclude, therefore, that only the interactions of molecules of type i with their immediate environment are important in Eq. 2, and the equation can now be used in two ways. (i) Analytical data for well-defined model systems can be used to evaluate the potential differences $\mu^{o}_{ia} - \mu^{o}_{ib}$ for appropriate differences. (ii) Conversely, we can estimate differences in μ^{o}_{i} between states that involve environments similar to those of model systems and predict the relative concentrations X_{ia} and X_{ib}.

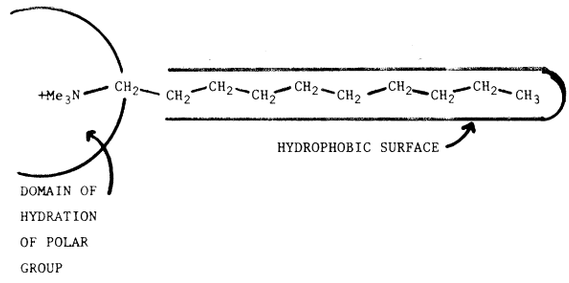

Fig. 1. Schematic diagram of an amphiphilic solute in aqueous solution. The strong attractive forces between the polar end and the surrounding water molecules probably neutralize the CH_2 group immediately adjacent to the polar group.

Hydrophobic Effect

Hydrophobic forces are not the only forces that can make large contributions to differences in unitary potential, even in the water-bathed environment of biological structures. They are not involved in the structure of bone and probably play only a minor role in the organization of the nucleus. They are important in the folding of proteins to produce compact three-dimensional structures, but other forces are equally important, especially in stabilizing a unique structure for each individual protein and making it relatively rigid and proof against deformation. I have chosen to emphasize hydrophobic forces in this article because they lead to structures that are not rigid and are thus uniquely suited for the first critical steps in the organization of living matter, where deformability is not only a virtue, but very likely a necessity.

The phenomenological definition of the hydrophobic effect (*5*) begins with the fact that hydrocarbon molecules have a much higher solubility in liquid hydrocarbons (or most other organic solvents) than they do in water. The unitary potential in the nonaqueous solvent (μ^{o}_{HC} if the solvent is liquid hydrocarbon) is more negative than that in water (μ^{o}_{w}) by several kilocalories per mole. The same phenomenon is observed with polar derivatives of hydrocarbons, where the difference in unitary potentials appears to be an additive function of the constituent groups. The contribution from the polar group favors the aqueous medium, but the hydrocarbon portion favors the nonaqueous medium, and its contribution to the difference between μ^{o}_{HC} and μ^{o}_{w} is essentially the same as it would be for a similar structural moiety within a pure hydrocarbon molecule. For linear alkyl chains the contribution to $\mu^{o}_{HC} - \mu^{o}_{w}$ is -850 calories per mole per CH_2 group and -2000 calories per mole per CH_3 group at 25°C (*5–9*). Nonlinear alkanes are somewhat less hydrophobic than their linear isomers because they are more compact and have a smaller area of contact with the solvent. Results for linear, branched, and cyclic alkyl chains can be accommodated by a single expression if the hydrophobic effect is considered as proportional to the surface area of the cavity created in the aqueous solvent by the hydrocarbon moiety (*10–13*).

For the purpose of the present discussion, the precise numbers are less important than the underlying mechanism from which the hydrophobic effect derives its name. The common mechanism for solvent preferences is based largely on solute-solvent attractive forces. Ionic and polar solutes are more soluble in water than in liquid hydrocarbon because there are strong attractive forces between the solute and water molecules, and polar substituents on alkyl chains make a positive contribution to $\mu^{o}_{HC} - \mu^{o}_{w}$ for the same reason. In the case of alkyl chains, however, solute-solvent attractive forces are weak both in the hydrocarbon environment and in the aqueous medium; in fact, the dipole-induced dipole attraction between H_2O and a CH_2 group may be slightly stronger than the attraction produced by dispersion forces between CH_2 groups. The large negative values of $\mu^{o}_{HC} - \mu^{o}_{w}$ for hydrocarbon solutes are due to the strong cohesive forces between water molecules and the fact that the network of H_2O-H_2O bonds is isotropic. Alkyl chains are literally squeezed out of the aqueous medium.

The basis for this distinction has long been known and has been explained eloquently in the past (*14–16*). However, because the absence of strong attractive forces between alkyl chains will be very important in the subsequent discussion, and because there has not been complete acceptance of the concept of hydrophobicity (*17*), I will note that the distinction is based on three separate lines of evidence.

1) Our knowledge of intermolecular forces: attractive forces between hydrocarbon groups or between them and other hydrophobic solutes, such as the inert gases, are the weakest such forces known.

2) Thermodynamic parameters other than the chemical potential: the partial molal heat capacity is especially compelling, as it can be measured separately in the organic solvent and in water, and it is the value in water that is anomalous to an extraordinary degree.

3) The indifference of hydrocarbon solutes to most organic solvents; for example, μ^{o}_{i} values in CCl_4 are virtually identical to those in hydrocarbon solvents. Water has an almost unique place as an extremely poor solvent for hydrocarbon solutes.

Minor details. Numerical values for the hydrophobic free energy have a small measure of uncertainty because it is not possible to correct experimental data for the effects of nonideal mixing in the organic phase. The experimental results on which the numbers are based do not include data for large complex fused rings: a very careful study with highly purified cholesterol (*9*) gave an anomalous value for $\mu^{o}_{HC} - \mu^{o}_{w}$, apparently not as the result of nonideal mixing statistics, which suggests that fused rings may be somewhat less hydrophobic than other molecules with the same surface area. Another factor affecting numerical calculations is the likelihood that the CH_2 group proximal to a polar group in an amphiphilic molecule does not contribute to hydrophobicity because, as illustrated by Fig. 1, water molecules in its vicinity are preempted by the attractive forces for the polar group (*18*). None of these considerations have any effect on the conclusions reached in this article.

Organizational Drive

The organizational drive of the hydrophobic effect asserts itself whenever there is in an aqueous medium a sufficiently high concentration of amphiphilic molecules (or ions) containing a polar or charged group at one end, attached to a relatively large hydrocarbon moiety, as in Fig. 1. The opposing thermodynamic preferences of the two ends of such a

molecule are most simply satisfied by self-association to form an aggregate with the hydrocarbon chains in the middle, avoiding contact with water as much as possible, and the hydrophilic polar groups at the surface, as illustrated by Fig. 2. The particle is called a micelle, and it typically contains on the order of 100 molecules per particle. Formation of a micelle is therefore a highly cooperative process, depending on a very high power of the monomer concentration. This means that there is a critical concentration (strictly speaking, a narrow concentration range) below which no micelles exist and above which virtually all added amphiphile enters the micellar state. The critical concentration depends chiefly on the size of the hydrophobic moiety, ranging from close to 1 molar for derivatives with hexyl chains to less than 10^{-9} molar for biological phospholipids.

The size and shape of micelles is determined by a combination of geometric and thermodynamic factors (*19*). One of the geometric factors is the surface/volume ratio, which for any reasonable assumed micelle shape has to decrease as the size increases; in other words, as the number of alkyl chains in the hydrocarbon core of the micelle becomes larger, the surface area per emerging chain has to become smaller. The second geometric factor, evident from Fig. 2, is that one dimension of a micelle core is restricted: it cannot be larger than the length of two alkyl chains, one approaching from each side. Moreover, the alkyl chains are normally in a liquid state; that is, their length is less than that of fully extended chains (*20*). The limited dimension calculated on this basis is about 24 angstroms for two dodecyl chains and about 30 angstroms for the chains in the C_{16} to C_{18} range normally found in phospholipids. These are small dimensions: if micelles were spherical with the diameter of the hydrophobic core equal to the limiting dimension, one could accommodate no more than 20 monomers with dodecyl chains in one micelle, or no more than 32 monomers with chains in the C_{16} to C_{18} range. Experimentally observed aggregation numbers are much larger than this.

Surface area calculations suggest why these small spherical micelles are thermodynamically unstable. Their surface/volume ratio would be large, and the area per emerging chain would be > 100 Å^2. This means that there would be considerable open space between head groups with contact between water and hydrocarbon, so that the hydrophobic drive force would not be optimally satisfied. Any increase in size would decrease

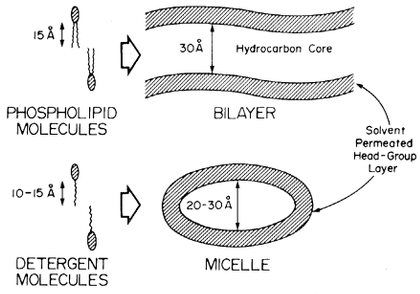

Fig. 2. Self-association of detergents and phospholipids to form micelles or planar bilayers, both shown in cross section. Most micelles are disklike and, being fluid, probably do not have a unique regular shape (*22*).

the area per emerging chain. Growth as a sphere being impossible, there are essentially two alternatives. One dimension can be kept fixed at the limited value and growth allowed in two perpendicular directions, forming an overall disklike structure; or two dimensions can be kept fixed and growth allowed along only one dimension, forming a cylindrical or rodlike particle. As shown in Table 1, a disklike structure represents the more efficient way to reduce the area and is therefore thermodynamically favored. Small micelles are, in fact, usually disklike, as demonstrated by measurements of their hydrodynamic properties (*19, 21, 22*).

It should be noted that the surface area of a micelle may not become too small because of repulsion between hydrated head groups. The optimal micelle size (on the order of 100 for dodecyl chains) seems to be reached in Table 1 when the area per head group is about 60 to 65 Å^2.

Table 1. Surface areas for micelles formed from dodecyl derivatives with a single alkyl chain per molecule as a function of aggregation number. The areas are calculated for micelles with a smooth hydrophobic core (with no allowance for surface corrugations) at 1.5 Å from the core surface. The CH_2 group adjacent to the polar group is assumed to remain in the aqueous medium.

Assumed geometry	Aggregation number	Area per chain = area per head group (Å^2)
Largest possible sphere	20	106
Disk with rounded edge*	50	76
	100	61
	200	51
Oblate ellipsoid*	100	64
Cylinder with caps†	100	72
Prolate ellipsoid†	100	78

*Two different geometric models that may be used to estimate areas of disklike micelles. The oblate ellipsoid is a poor model for very large aggregation numbers. †Two different geometrical models that may be used to estimate areas of rodlike micelles.

Calculations in Table 2 for longer alkyl chains indicate that a higher aggregation number is needed to attain the same area, and this agrees with the effect of alkyl chain length on experimentally measured micelle size.

Thermodynamics of Micelle Formation

Rigorous thermodynamic equations (*19, 23*) can be obtained in either of two ways. One can consider micelles of aggregation number m, with m ranging from 2 to ∞, to be separate chemical species, and then work in terms of the chemical equilibria $mZ \rightleftarrows Z_m$, where Z represents a monomeric molecule (*23*). Alternatively, one can consider the micelles to be separate phases and apply the equilibrium condition that the chemical potential of an amphiphile molecule in a micelle of size m ($\mu_{\mathrm{mic},m}$) is the same in all micelles and equal to its chemical potential as a monomer in aqueous solution (μ_{w}). If the latter approach is used (both approaches give the same result), the expression used for the chemical potential in Eq. 2 is correct only for μ_{w}. The corresponding term for $\mu_{\mathrm{mic},m}$ must take account of the facts that it is whole micelles and not individual amphiphile molecules that are mixed with solvent and that $\mu_{\mathrm{mic},m}$ represents $1/m$ of the chemical potential of the micelle. One obtains

$$\mu_{\mathrm{mic},m} = \mu^{\circ}_{\mathrm{mic},m} + (RT/m) \ln (X_m/m) \quad (3)$$

and, for the equilibrium condition

$$RT \ln (X_m/m) = m(\mu^{\circ}_{\mathrm{w}} - \mu^{\circ}_{\mathrm{mic},m}) + mRT \ln X_{\mathrm{w}} \quad (4)$$

where X_m is the mole fraction of amphiphile in micelles of size m, and Eqs. 3 and 4 apply separately to all possible values of m. If one can estimate the unitary potential difference ($\mu^{\circ}_{\mathrm{mic},m} - \mu^{\circ}_{\mathrm{w}}$) as a function of m, one obtains a size distribution function as a function of X_{w} and from it the experimentally determinable average size and the critical concentration for micelle formation.

It is not possible to go into details of the calculation here. However, reasonable choices of the parameters that go into the calculation do lead to correct predictions of micelle size and critical micelle concentration for amphiphiles with different head groups, and they confirm the observation that a disklike shape is preferable to a rodlike shape and that a surface area of about 60 to 65 Å^2 per head group is appropriate for optimal stability (*24*). To illustrate what is meant by reasonable choices of parameters, I mention one parameter, the gain in uni-

Table 2. Surface areas for diacyl phospholipids with chains in the hexadecyl to oxadecyl range. The conditions are as described in the legend of Table 1.

Hydrocarbon chains per particle	Area (Å²)	
	Per alkyl chain	Per head group
Disk with rounded edge		
50	93	186
100	74	148
200	61	122
1,000	45	90
10,000	36	72
100,000	34	68
*Spherical vesicle**		
1,000	37	74
10,000	33	66

*Experimental aggregation numbers for single-walled vesicles formed spontaneously by sonication are about 2000, corresponding to 4000 hydrocarbon chains per vesicle.

tary potential from transferring an alkyl chain from water to the hydrocarbon interior of a micelle, which makes a major contribution to $\mu^0_{mic,m} - \mu^0_w$. The hydrocarbon core of a micelle cannot be regarded as equivalent to bulk liquid hydrocarbon because it is a two-dimensional rather than a three-dimensional liquid. Individual alkyl chains are flexible and can diffuse freely in two dimensions, but they cannot tumble. This restriction must make the unitary potential more positive in a micelle than in bulk hydrocarbon. To obtain agreement with experiment in the dependence of critical micelle concentration on alkyl chain lengths, for amphiphiles with a variety of head groups, one must set the contribution per CH_2 group at -710 cal/mole, a reasonable value in relation to the figure of -850 cal/mole given earlier for bulk hydrocarbon. (The terminal CH_3 group of an alkyl chain is presumably as free in a micelle as in bulk liquid, but its contribution is one of a number of invariant parameters, only the sum of which enters into the theoretical calculation.)

Phospholipids

Phospholipid molecules contain two hydrocarbon chains per head group instead of the single hydrocarbon chain of amphiphiles that form small micelles (Fig. 1). This difference alone accounts for the formation of extended phospholipid bilayers, as shown by the data in Table 2, which are for an alkyl chain length typical of those found in natural phospholipids. Suppose, for example, one were to form a small micelle containing 200 hydrocarbon chains, which would give a surface area that would be in the optimal range if there were one head group per chain. A micelle of this size formed by diacyl phospholipids would, however, have only 100 head groups, and the area per head group would be twice the area per emerging chain. This is very far from the optimal area, and an area in the optimal range can be approached only if the disk-shaped micelle is grown to virtually infinite size, where it becomes effectively an extended bilayer, as illustrated in Fig. 2.

Another way to reduce the average area, which does not require as high a degree of aggregation, is to form a closed vesicle with an internal water-filled cavity, as illustrated by Fig. 3. Counting both inside and outside areas, the average area for a particular aggregation number is less for such a vesicle than for a circular disk with a rounded edge. Vesicles are, in fact, formed spontaneously whenever phospholipids are dispersed in an aqueous medium. They tend to be multiwalled rather than single-walled when the concentration of lipid is initially large, but single-walled vesicles are readily obtained under appropriate conditions, as when the lipid suspension is stirred vigorously by sonication (*25–27*).

Compartmentalization: The First Step in Biological Organization

The formation of vesicles is the first essential step in biological organization; it is, in effect, a beginning in the definition of a living cell. It occurs spontaneously, simply as a result of the drive toward thermodynamic equilibrium, once the appropriate molecules (phospholipids) have been synthesized. From the point of view of thermodynamics, however, there is an element of paradox. Because the vesicle wall contains a core of liquid hydrocarbon that is 30 Å thick, it is an effective barrier to ions, hydrophilic metabolites, proteins, nucleic acids, and so on. Communication between the inside and the outside of the vesicle is thus prevented, and there is no longer a pathway for equilibration between them. In other words, lipid molecules, in striving for their own thermodynamic equilibrium, have created the kind of structure necessary to prevent equilibration between most water-soluble substances. The chemical composition of the internal space need not be the same as that of the external environment, and the reactions that go on are, in principle, independent of the external environment as long as the vesicle walls remain intact.

A sealed-off compartment is not yet

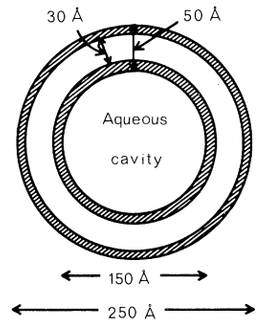

Fig. 3. Schematic diagram of a phospholipid vesicle. The dimensions given are close to the experimental dimensions of egg yolk lecithin vesicles formed by sonication (*25*).

representative of a cell or of the kind of intracellular compartments that exist within the cells of higher organisms. It is necessary to have controlled access for metabolites to provide energy within the compartment; building blocks to synthesize nucleic acids and proteins (and more phospholipid to permit cell growth and division); ion channels to conduct nerve impulses; recognition sites in the outer surface of cell membranes to organize multicellular structures or act as receptors for circulating hormones; mechanisms to maintain ion gradients and to secrete hormones, enzymes, and neurotransmitters; and sometimes channels to permit communication between adjacent cells. These additional structural elements have to have extraordinary specificity. One might think of them as analogous to stopcocks that provide access to a laboratory flask, but they need not only to have the mechanical ability to control flow but also to be the brains and hands of the experimenter, to determine precisely what reagent to add and when to do it. The only molecules known to be capable of such specificity are proteins, and membrane-bound proteins and glycoproteins that carry out these vital functions have been discovered in large number in recent years. In the following section I will show that the ultimate location of these proteins in membranes is probably also under thermodynamic control, and that the hydrophobic effect again plays a critical role in the process.

Protein Folding and Membrane-Bound Proteins

It is well established that the three-dimensional structure of proteins is determined by the sequence of amino acids alone: an unfolded linear polypeptide chain spontaneously folds to the same final structure in vitro as it does in the living cell (*28, 29*). The final structure is

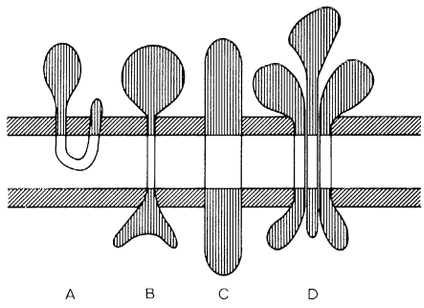

Fig. 4. Schematic representations of membrane-bound proteins. The shaded portions have hydrophilic surfaces; the unshaded portions have hydrophobic surfaces in contact with the hydrocarbon core of the bilayer. Proteins may lie entirely on one side of the membrane (A) or may have portions exposed on both sides (B to D). The hydrophobic part of the protein may represent only a small part of the whole (B). Membrane proteins, like other proteins, may be composed of more than one like or unlike subunit, held together by noncovalent forces (D).

largely under thermodynamic control, subject as usual to certain constraints, the major one being kinetic. The process begins with the folding of a particular portion of the polypeptide chain to its own thermodynamically stable state, and that portion may remain intact during subsequent stages of the process, especially if they are fast. Some structural states may thus be kinetically inaccessible during the search for a final equilibrium structure. How frequently (if ever) such kinetic constraints prevent attainment of a hypothetical true equilibrium state of lower free energy than the native state is not known.

The forces involved in protein folding are also well understood (*16*). Hydrophobic forces play an important role (*30*), but steric constraints, hydrogen bonds, and hydration energies of charged groups are equally important. For many water-soluble proteins the end result is a compact globular particle with charged groups invariably at the surface and hydrophobic groups mostly on the inside, avoiding contact with water as best they can. Hydrophobic groups are derived from amino acids with hydrocarbon side chains and are considerably smaller than the hydrocarbon chains of phospholipids or of micelle-forming amphiphiles. Furthermore, the amino acids with hydrophobic side chains are usually interspersed with hydrophilic amino acids in the sequence. The exact structures that are known (*31*) (for about 30 or 40 water-soluble proteins) thus do not contain conspicuous large internal domains consisting purely of hydrocarbon, and the overall structures tend not to be significantly deformable. The peptide groups of the polypeptide backbone are intrinsically hydrophilic, but they appear to be stable away from contact with water if they can form hydrogen bonds with each other, such as occur in the well-known α-helix and the parallel or antiparallel β-sheet structures. The existence of such structures within the folded protein further enhances the structural rigidity.

How are membrane-bound proteins different? In discussing this I will consider only the so-called intrinsic or integral proteins (*32, 33*)—that is, proteins that traverse the phospholipid bilayer or are firmly anchored in it, with intimate contact between protein and hydrocarbon chains, as illustrated schematically by Fig. 4. All proteins that play the kind of functional role envisaged above are in this category. Amino acid compositions are known for many of them, and it is clear that they are constructed from the same amino acids as water-soluble proteins; moreover, they do not have a significantly different overall composition in terms of the fractions of the amino acids that are hydrophilic and strongly hydrophobic. In particular, they have about the same fraction of ionic side chains as water-soluble proteins, and these side chains have to be in close contact with water. Even in the absence of actual structural information, it is therefore certain that much of the protein surface must ordinarily project outside the bilayer into the adjacent medium, as in the schematic models of Fig. 4. The existence of these external regions is appropriate from a functional point of view, because all the functions envisaged involve recognition of extramembranous ions or water-soluble substrates, hormones, and so on.

There is substantial evidence that the location of membrane proteins in membranes is under thermodynamic control; proteins have been extracted in various ways, and normally functioning protein-containing membranes have been reconstituted. Moreover, most membrane proteins are virtually insoluble in aqueous salt solutions but can be solubilized in appropriate benign detergents without changes in structural parameters that can be measured (for instance, overall compactness and circular dichroism spectrum) (*34*). Any part of their function that can be assayed in solution, such as the specific ion-stimulated adenosinetriphosphatase activity of adenosine triphosphate–driven ion pumps, is generally preserved under these conditions. Since detergent micelles offer the same kind of local environment to the protein as a phospholipid bilayer—that is, a narrow layer of hydrocarbon bounded on both sides by an aqueous medium (Fig. 2)—it is reasonable to infer that this kind of environment is thermodynamically favored. If the principle that amino acid sequence determines three-dimensional structure is preserved (there is no reason to suppose that it is not), membrane proteins must differ from water-soluble proteins at the primary sequence level. Their amino acid sequences must direct spontaneous folding to three-dimensional structures that contain substantial hydrophobic surfaces, structures that would have a high chemical potential in an aqueous medium and a much lower one if the protein comicellizes with other substances, whether they are detergents forming small micelles or lipids forming more extended bilayers (*35*).

Only two or three membrane proteins have known amino acid sequences (none of them is involved in transmembrane communication), and the data support the idea that membrane association is the

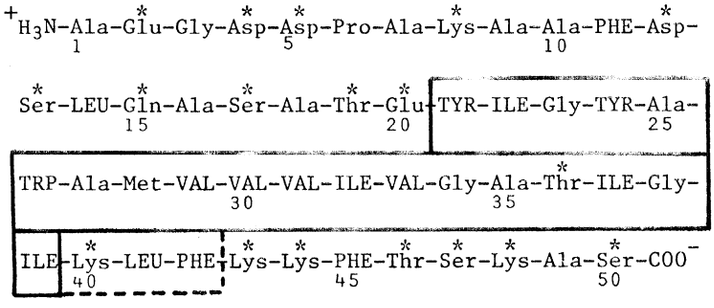

Fig. 5. Amino acid sequence of the coat protein of bacteriophage fd (*36, 47*). Amino acids with hydrophobic side chains are shown by capital letters, and those with strongly hydrophilic side chains by an asterisk. The presumed membrane-bound region is enclosed by solid lines; it may include residues 40–42 because the lysine side chain is long enough to allow the α carbon of residue 40 to be within the hydrocarbon core, while the charged NH_3^+ group lies beyond the next two hydrophobic side chains on the outside.

result of specialized sequences. One established sequence is for the coat protein of the filamentous bacteriophage of the fd type (*36*), shown in Fig. 5. It contains an unbroken sequence of about 20 hydrophobic residues, with a single threonine the only midly hydrophilic amino acid in the sequence. The protein is bound to the membrane of the host bacterium (*Escherichia coli*) during phage morphogenesis, and this segment of sequence is responsible for the binding and has been shown to be the membrane-bound portion in reconstituted lipid vesicles (*37*). Another established sequence is that of cytochrome b_5 from liver microsomal membranes. This protein lies entirely on one side of the membrane (as in Fig. 4A), and it has a three-dimensional structure composed of independently folded domains, which can be isolated as separate fragments after mild proteolysis (*38, 39*). The major fragment is water-soluble and contains the active site of the protein. A 40-residue fragment, relatively rich in hydrophobic amino acids, is responsible for association with the membrane. Instead of a single long hydrophobic sequence it has two shorter ones of eight residues apiece, separated by about ten polar residues (*40*), suggesting that attachment to the membrane is by two shallow U-shaped inserts.

Aqueous Channels Through Membranes

The short hydrophobic sequences just discussed are appropriate for forming solid inserts for anchoring receptors or recognition proteins to a membrane, but are not suited for transmembrane proteins involved in the transport of ions or polar molecules. These proteins must have more sophisticated structures that include provision for the passage of hydrophilic substances through the membrane. Figure 6 shows a purely speculative structure that could accomplish this. This structure might be formed by an alternating sequence in which there is a strongly hydrophilic residue at every third or fourth position in an otherwise hydrophobic stretch of amino acids. Such a sequence could form helices with a predominantly hydrophobic surface, but possessing one hydrophilic edge (*41, 42*). Six or more such helices could combine to form a structure with a hydrophobic surface, but with an aqueous channel down the middle. The individual helices could be parts of the same polypeptide chain, as in bacteriorhodopsin (*43*), or the overall structure could be an oligomer of identical chains, each contributing one helix.

Unique Fitness of Hydrophobic Forces

The structural organization I have described (solely in terms of the final equilibrium state; the means of arriving at this state represent a separate problem, considered later) consists of three stages: formation of phospholipid bilayers and vesicles, folding of proteins to their native structures, and insertion of appropriately structured proteins into bilayers. The thermodynamic driving force for the first and last stage is a repulsive force acting on the separated elements and not a preferential attraction. I believe this to be an essential feature of the process. Because of it, micelles and bilayers are fluid. There are no strong attractive forces between alkyl chains in the hydrocarbon core and no preferences for particular neighbors: micelles and bilayers are formed from mixtures of amphiphiles as readily as from pure ones. This indifference to local arrangement is crucial for detergency: soap and detergent micelles indiscriminately absorb any molecule that has a hydrophobic piece. Incorporation of membrane proteins into membranes can be thought of as a form of detergency that comes into play whenever a protein molecule with a hydrophobic piece has been synthesized in the cell.

Moreover, fluidity per se is vitally important for life processes (*33*). Cells have to be capable of deformation, as part of locomotion, for example, or when squeezed by neighboring cells. They have to be capable of instantly resealing if accidentally nicked at some point on the surface.

These essential properties of membranes—deformability and accommodation to appropriate insertions—would be virtually impossible to achieve if the permeability barriers that represent the first stage of compartmentalization were to be based on specific attractive forces, "bricks and mortar," that normally lead to rigid inflexible structures (illustrated by protein molecules, where internal hydrogen bonds play an important role). The fact that nature uses the hydrophobic force as the factor that creates compartments and the connectors between them is not an arbitrary choice. It is difficult to imagine it done in any other way.

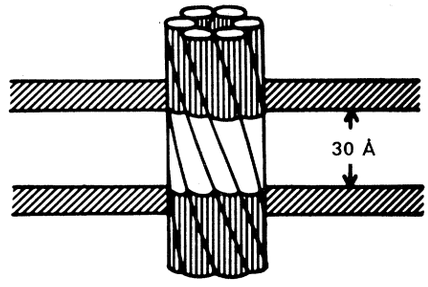

Fig. 6. Hypothetical model for the formation of an aqueous channel across a phospholipid bilayer. The twisted cylinders are helical segments of a polypeptide chain. Shaded portions of the surface are hydrophilic; unshaded portions are hydrophobic. [This is patterned after a proposal by Inouye (*42*) for a somewhat different kind of protein.]

Concluding Remarks

In this article I suggest that a reasonable initial approach to an understanding of the organization of living matter, even in the most complex multicellular organisms, is to divide the process into two parts: (i) biosynthesis of the appropriate molecules and its control and (ii) assembly of these molecules into organized structures. The second part of the process appears to be largely under thermodynamic control; that is, organized structures result simply from the search of each molecule for its position of lowest chemical potential.

I have focused on the creation of cell membranes and means of communication between the two sides, but there is every reason to believe that the same principle applies to the extension of cells in space, the creation of intercellular contacts, and other aspects of biological organization. That virus particles are self-assembling is already well established (*44*). The contribution of the hydrophobic effect to the chemical potential would, of course, be much less important for these other aspects of organization than it is for the formation of membranes. In fact, we can expect directed polar bonds to play a dominant role whenever the desired end result is a rigid structure, as exemplified by the role of rigid networks of hydrogen bonds in the formation of the ordered structures of proteins.

Thermodynamic analysis serves to simplify and clarify one's view of a subject. It answers some questions, but may be even more useful in defining problems for future investigation. In fact, in the development of physical chemistry, identification of an equilibrium state (and establishment that it is the equilibrium state) has usually been followed by investigation of the mechanism by which the equilibrium state is attained. The same must be true in biology, and an intriguing question, which is currently receiving much attention from cell biologists, concerns proteins that traverse membranes and have exposed hydro-

philic portions on both sides (Fig. 4). The phospholipid bilayer, once formed, is impermeable to ionic molecules, but these proteins are synthesized on one side of an already existing bilayer, and some of their ionic amino acid side chains have to pass through the bilayer to reach the other side. The current status of work on this problem has been reviewed (*45*). A similar question can be asked with reference to the entry of nucleic acids into cells, such as occurs on infection by viruses, and to the transfer of newly synthesized phospholipid molecules to the external half of a bilayer (*46*).

It is noteworthy that these questions do not arise in relation to an unexpected failure to attain an equilibrium state. On the contrary, they seek to explain the existence of a state close to equilibrium where there are known physical constraints that would be expected to block the approach to equilibrium.

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46. A more subtle problem that has not been actively pursued has to do with differences in acyl chain composition of lipids from different membranes. About half of them are generally unsaturated, which serves to prevent crystallization of the hydrocarbon core of the bilayer and keep it fluid. But no known purpose is served by such deviations from the norm as the presence of docosohexanoyl chains (six unconjugated double bonds) as the predominant unsaturated chains in retinal rod outer segment membranes. Similarly, there is no obvious functional need for the presence of cholesterol in the membranes of eukaryotic cells.
47. Abbreviations: Ala, alanine; Glu, glutamic acid; Gly, glycine; Asp, aspartic acid; Pro, proline; Lys, lysine; Phe, phenylalanine; Ser, serine; Leu, leucine; Gln, glutamine; Thr, threonine; Tyr, tyrosine; Ilc, isoleucine; Trp, tryptophan; Met, methionine; and Val, valine.

48. I am grateful to J. A. Reynolds for numerous helpful suggestions in the preparation of this article, and to the National Science Foundation for research support.

The Physics Interviewing Project: A Tour of Interviews in Asia

Earl Callen and Michael Scadron

The American professor stood on a rickety laboratory stool, a 1-rupee coin in one extended hand, an iron pendulum bob in the other. The Asian physics student, a Master of Science candidate and an honors graduate with distinction, stood insisting that "heavy objects hit the ground first because they are heavier." Another scene in another country: the student now before us was an undergraduate, but older than most. He knew and understood well much of what is taught in an American graduate physics curriculum; he had taught himself physics while in hiding during the 1970–1972 Bangladesh war. The student had few formal qualifications, but he was very good.

This was the Physics Interviewing Project (*1*). The incidents indicate the nonuniformity and sometimes unreliability of degrees, honors, and rankings. And they point up the value of a personal interview, which is why there is a Physics Interviewing Project. The Project, organized about a decade ago by M. J. Moravcsik of the University of Oregon, aids foreign students seeking admission and support from Western graduate physics

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