Minireview

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Detergents as Tools in Membrane Biochemistry*

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R. Michael Garavito‡ and Shelagh Ferguson-Miller§

From the Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, Michigan 48824-1319

Detergents are invaluable tools for studying membrane proteins. However, these deceptively simple, amphipathic molecules exhibit complex behavior when they self-associate and interact with other molecules. The phase behavior and assembled structures of detergents are markedly influenced not only by their unique chemical and physical properties but also by concentration, ionic conditions, and the presence of other lipids and proteins. In this minireview, we discuss the various aggregate forms detergents assume and some misconceptions about their structure. The distinction between detergents and the membrane lipids that they may (or may not) replace is emphasized in the most recent high resolution structures of membrane proteins. Detergents are clearly friends and foes, but with the knowledge of how they work, we can use the increasing variety of detergents to our advantage.

Over the past decade, our understanding of the structure and function of membrane proteins has advanced significantly as well as how their detailed characterization can be approached experimentally. Detergents have played significant roles in this effort. They serve as tools to isolate, solubilize, and manipulate membrane proteins for subsequent biochemical and physical characterization. Many of the successful methods for reconstituting (1) and crystallizing (2–4) membrane proteins rely on the unique behavior of detergents. Although many new detergents are now available for use with membrane proteins, their behavior in solution and in the presence of protein may limit their use with specific experimental techniques. Hence, the choice of detergent and experimental conditions will have an enormous impact on whether a technique can be successfully applied to a specific membrane protein. A clear understanding of basic detergent behavior and of the structure of micelles and protein-detergent complexes is thus crucial for membrane biochemists.

In this minireview, we will briefly discuss the basic aspects of detergent physical chemistry that affect membrane proteins and their manipulation in the context of the new information about membrane protein structure and function. The reader is directed to comprehensive reviews by Helenius and Simons (5), Tanford and Reynolds (6), Helenius *et al.* (7), Kühlbrandt (4), and Zulauf (8), which cover the action and behavior of detergents from a biochemical viewpoint. Excellent monographs by Tanford (9) and Rosen (10), as well as a review by Wennerström and Lindman (11), describe the physical chemistry of detergents and surfactants in detail.

Detergents and Lipids as Surfactants

Detergents are surface-active molecules that self-associate and bind to hydrophobic surfaces in a concentration-dependent manner (8, 10, 11). The amphipathic character of detergents is evident in their structures (Fig. 1*a*), which consist of a polar (or charged) head group and a hydrophobic tail. Most detergents fall into one of three categories depending on the type of head group: ionic (cationic or anionic), nonionic, and zwitterionic. The behavior of a specific detergent is dependent on the character and stereochemistry of the head group and tail.

In the broader sense, detergents and lipids are both surfactants. What distinguishes one from the other are the concentration regimes for self-association and the kinds of multimolecular structures each can make. The problem of isolating native membrane proteins from lipid bilayers and then subsequently manipulating them is, in essence, a problem of dealing with mixed surfactant systems. The most common question about detergent use is whether a "magic bullet" detergent exists. The simple answer is no, but successful strategies for detergent use do exist. The key to a successful experiment is to understand how detergents and lipids impact the physical nature of a protein-detergent-lipid complex and its behavior.

The Micelle: What Is It?

Detergent monomers in aqueous solutions are involved in two kinds of basic phase transitions. First, monomers can crystallize in aqueous solution (10), although the majority of detergents used in membrane biochemistry do not (4–7). Second, detergent monomers self-associate to form structures called *micelles* (8, 10, 11). At a broad threshold of monomer concentration called the critical micelle concentration (CMC)[1] (Fig. 1*b*), self-association occurs and micelles form. Ideally, the concentration of detergent monomers stays constant above the CMC as more detergent is added to the solution; only the concentration of micelles increases (12). When the concentration exceeds the CMC, a detergent becomes capable of solubilizing hydrophobic and amphipathic molecules, such as lipids, into mixed micelles or micellar aggregates (10). Moreover, the complete and stable solubilization of many integral membrane proteins generally occurs above the CMC, as the detergent associates with the hydrophobic surfaces of membrane proteins to create water-soluble protein-detergent complexes (PDCs) (13–15).

Micellarization is a common phenomenon with many surfactants. The average size and shape of micelles depend on the type, size, and stereochemistry of the surfactant monomer (10, 11, 16) as well as the solvent environment. The size of a micelle can be described by its average molecular weight, hydrodynamic radius, and aggregation number (the average number of monomers per micelle). The physical and chemical characteristics of a detergent determine micelle size and shape as well as the size and shape of the detergent layer on a protein.

Detergent monomers are often assumed to form relatively uniform surfaces in micelles and in PDCs. This misconception arises from our simplistic cartoons of spherical micelles, wherein the hydrophobic tails, in a *trans* configuration, are shown extending toward the center of the micelle (Fig. 2*a*). This geometrically impossible picture (8, 9) obscures some important insights into how the size, shape, and behavior of a micelle (or a PDC) are dependent on detergent packing. More realistic pictures of a detergent micelle

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‡ To whom correspondence may be addressed. Tel.: 517-355-9724; Fax: 517-353-9334; E-mail: garavito@msu.edu.

§ To whom correspondence may be addressed. Tel.: 517-355-0199; Fax: 517-353-9334; E-mail: fergus20@msu.edu.

[1] The abbreviations used are: CMC, critical micelle concentration; PDC, protein-detergent complex; LC, lower consolute; β-OG, β-D-octyl glucoside; UC, upper consolute.

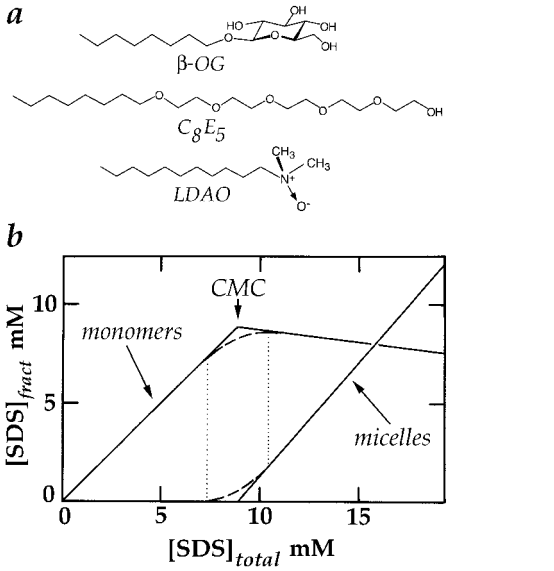

FIG. 1. **Detergent structure and micellarization.** Detergent monomers of β-OG, octyl-pentaoxyethylene (C_8E_5), and lauryl-dimethylamine-oxide (*LDAO*) are shown in *a*; each consists of a polar head group and *N*-alkyl tail. In *b*, the change in concentration ($[SDS]_{fract}$) of monomer and micellar fractions *versus* the total detergent concentration is shown for SDS. The CMC is the threshold detergent concentration where micelles begin to form. However, the CMC is not truly a sharp boundary, as the physical changes being followed (light scattering, surface tension, etc.) show broad transitions around the CMC (*dashed lines*). Thus, the CMC is often the midpoint of a concentration range (*dotted lines*). The figure shown in *b* was adapted from Ref. 12 (reprinted with permission; copyright 1980 American Chemical Society).

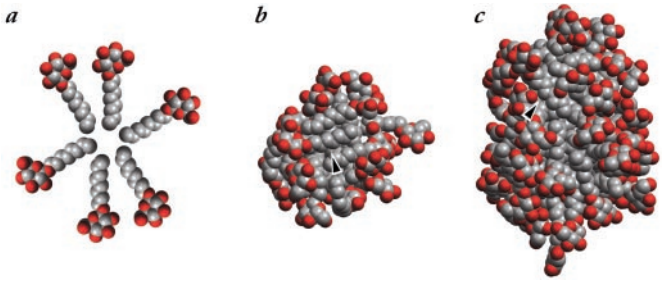

FIG. 2. **Space filling models of β-D-octyl glucoside micelles: classical representation in *a*, 20-monomer micelle in *b*, and 50-monomer micelle in *c*.** The micelles shown in *b* and *c* were derived from 40 ns molecular dynamics simulation data (17) and have nonspherical and nonuniform shapes. The polar portions of the detergents (oxygen atoms are *red*; carbon atoms, *gray*) do not cover completely the micelle surface. Hence, substantial portions of the core are exposed to bulk solvent, including alkyl chains lying along the micelle surface (*arrowheads*).

(Fig. 2, *b* and *c*) have the hydrophobic tails packing in a much more disorganized but compact fashion (17, 18). Two consequences of micelle structure are now clearly evident: 1) the micelle surface is quite rough and heterogeneous in character and 2) not all hydrophobic tails are buried or point toward the center of the micelle. Hence, micelle radii are about 10–30% smaller than the fully extended length of the detergent monomer (8), and many of the hydrophobic tails have considerable contact with water and solutes. Moreover, molecular dynamics studies (17, 18) also show that micelle shape is very dependent on aggregation number (Fig. 2, *b* and *c*) and that the concept of a "spherical" micelle really denotes only an *average* shape.

The concept of a compact, disordered micelle clearly suggests that monomer packing defects could radically affect the size, shape, and behavior of micelles. As lipids, other detergents, or amphipathic solutes are incorporated into the micelles of a pure detergent to form mixed micelles, packing defects may be introduced or, on the other hand, eliminated. By extrapolation, the bound detergents in a PDC are unlikely to be well ordered and efficiently packed. Perhaps the inability of certain detergents to solubilize or stabilize some membrane proteins arises from the unstable, defect-ridden packing of detergent monomers on the surface of the protein.

Another misconception is that micelles are static structures of uniform shape. The term monodisperse is often applied to colloidal systems to signify a uniform size and shape of a population of particles. For detergents, monodispersity is better perceived to be a *lack of detectable* heterogeneity in the *average* micelle size and shape (19). The experimental evidence suggests that micelles are quite fluid and rapidly exchange micellar components with the solvent (10, 11, 20, 21). Micelles of small detergents can exhibit dramatic fluctuations in micellar shape; they can deform, split, and fuse over time (10, 11, 17, 18). For some detergents, appreciable changes in micelle aggregation number, size, and shape may occur as the total detergent concentration rises (22, 23). Changes in micelle shape, from spherical to ellipsoidal or even rodlike, occur with many pure detergents (22, 23) but may be even more common when a detergent is mixed with another detergent, lipid, or protein (24).

Surfactant Phase Behavior

Self-association and crystallization are only two of many possible phase transitions that surfactant solutions may exhibit (8, 10). Phase diagrams of detergent behavior in aqueous solutions are generally simple for the nonionic detergents with *N*-alkyl tails of 8 carbons (Fig. 3). Nonionic and zwitterionic detergents with *N*-alkyl tails of 12 carbons or longer tend to exhibit much more complex phase behaviors (Fig. 3), where some phase changes involve micellar growth and/or fusion to form mesophases with distinct structural properties (8, 10, 16). One common detergent phenomenon is called the *cloud point* (8, 16), where a clear, homogeneous detergent solution turns turbid upon heating. The formerly single liquid phase (L_1) eventually separates into two immiscible solutions ($L_1' + L_1''$), one detergent-rich and the other detergent-poor. The boundary between the isotropic detergent phase and the co-existence of the two liquid phases (Fig. 3) is called a *consolute boundary* (8, 16). Bordier (25) recognized that this phase phenomenon could be exploited for membrane protein purification, and the technique of detergent phase separation is still used today (26).

The phase transitions exhibited by a particular surfactant are determined by its monomer structure (shape) as well as its chemistry (8, 16), *e.g.* its ionization state or capacity for hydration. Thus, changes in the solvent environment can also alter the nature of surfactant aggregation (8, 27). The mere addition of salts or polar solutes to a detergent solution can radically alter the phase behavior of a detergent system, causing phases to appear well below the relatively high detergent concentrations seen with the pure detergents (8, 16). The cloud point phase separation is a frequent problem during membrane protein crystallization (2–4) and is easily induced by a number of variables (*e.g.* detergent type, salt, temperature, and precipitant). For example, the octyl-oligooxyethylene (C_8E_m) detergents display a lower consolute (LC) boundary (Fig. 3). As the temperature rises, micelles aggregate into clusters (8, 23) until these clusters phase out to form a new aqueous, detergent-rich phase. The addition of salt also depresses the LC boundary to lower temperatures (8, 27). In contrast, the addition of polyethylene glycol to solutions of alkyl glycoside detergents, such as β-D-octyl glucoside (β-OG) and β-D-decyl maltoside, causes an upper consolute (UC) boundary to appear (Fig. 3). The take home lesson is that solution and environmental parameters affect not only the basic detergent phenomenon we rely on (micellarization) but also whether other detergent phases appear or not.

Mixed Micelles, Protein-Detergent Complexes, and Crystallization

What makes understanding surfactant phase phenomena so important to membrane biochemists is that the mere use of detergents with membrane proteins forces us to confront them, from protein isolation to crystallization to reconstitution. How a membrane protein behaves will be influenced by detergent-protein and detergent-detergent interactions, as well as interactions with any remaining lipid. Considering only detergents and lipids, it is known that mixed systems *will not behave* like solutions of the pure components (10, 11). Hence, changes in micelle shape and size, CMC, and phase behavior can all occur and they are not easily predicted, even for simple solutions containing two detergents.

The addition of a membrane protein to the mix further complicates matters. The fluidity and packing efficiency of the detergent

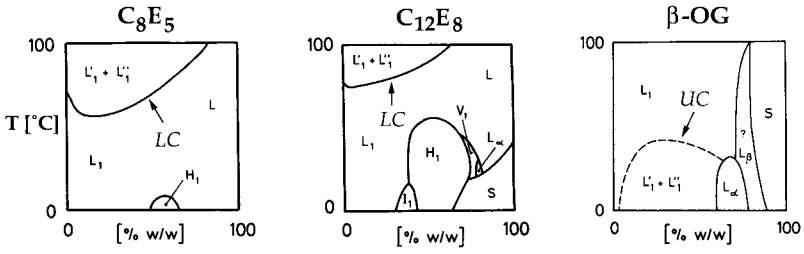

FIG. 3. **Temperature *versus* detergent concentration phase diagrams for octyl-pentaoxyethylene (C_8E_5), dodecyl-octaoxyethylene ($C_{12}E_8$), and β-OG.** Although the phase diagram for C_8E_5 is quite simple, the equivalent diagram for $C_{12}E_8$ shows several additional phases (see Refs. 8 and 16 for details). For C_8E_5 and $C_{12}E_8$, detergent phase separation is often seen under experimental conditions because salts and polymers may depress the LC boundary to below room temperature. $C_{12}E_8$ also exhibits the hexagonal H_1 phase (hexagonal packing of rodlike micelles) at 50% (w/w) mixture with water at 30 °C. At a threshold detergent concentration, bicontinuous cubic (V_1) and lamellar (L_α) phases are seen (8, 16). For β-OG in water, only the lamellar L_α and gel L_β phases are observed, aside from solid detergent (S). However, the addition of polyethylene glycol causes the appearance of an UC boundary, which rises with increasing polymer or salt concentration (2, 8). The phase diagrams were reproduced from Ref. 8 (with permission of CRC Press, Inc.).

monomers bound to the protein will affect the behavior and stability of the detergent layer. This may result in poor protein solubility and protein inactivation/aggregation. Thus, detergent behavior, during and after protein extraction from a bilayer, will impact the isolation (13, 14, 28), characterization (13, 15, 29), and stability (13, 30) of membrane proteins. When considering the added effects of other solvent components (salt, pH, etc.), seemingly small changes in experimental conditions may give rise to detergent effects not expected from the pure detergent.

How detergent behavior impacts the solubility, stability, and structure of PDCs is then important to know. For membrane protein crystallization, an early major emphasis was placed on creating simple, lipid-free PDCs (3, 4), using nonionic detergents that produced small, almost spherical micelles (8, 31) to control the shape, size, and behavior of the PDC. It was soon recognized that detergent-dependent phase transitions had an enormous impact on crystallization. Unwanted phase behavior could prevent crystal growth (32) and even denature protein (33). However, in many cases, crystal growth often occurred as conditions approached an upper or lower consolute phase boundary (3). Since then, much effort has focused on understanding the relationship between detergent-dependent phase behavior of the PDC and crystal growth (15, 29), as well as how the characteristics of the PDC can be altered by different detergents (2, 3, 31, 32) and the addition of small, amphiphilic consolutes (15, 34, 35).

The characterization of membrane protein crystals by single-crystal neutron diffraction and D_2O/H_2O density matching (36–39) has provided a wealth of information about the shape and structure of a PDC. For example, the structures of OmpF porin from *Escherichia coli* in different detergents and crystal forms revealed some interesting aspects about detergent behavior. Pebay-Peyroula *et al.* (36) studied the tetragonal crystal form of OmpF porin containing decyl-dimethylamine-oxide or β-OG. With decyl-dimethylamine-oxide, the PDC behaved as a "hard surface" complex (see Fig. 2 in Pebay-Peyroula *et al.* (36)), where the detergent layer appeared as a discrete and continuous torus about the protein. In contrast, the porin·β-OG complex revealed a partial fusion of the detergent torus with its neighbors (see Fig. 6 in Pebay-Peyroula *et al.* (36)). When Penel *et al.* (37) looked at the trigonal crystal form of OmpF porin containing octyl-hydroxyethyl-sulfoxide (see Fig. 4 in Penel *et al.* (37)), the detergent torus about each porin molecule had completely fused with its nearest neighbors to create a continuous detergent domain within the crystal. Clearly, detergents that should normally just produce small spherical or ellipsoidal micelles can be induced to form more complex structures at concentrations below 50% (w/w). Moreover, detergent-detergent interactions are often *an integral part* of the long range structure in membrane protein crystals.

If detergent interactions and structure play a role in membrane protein crystal growth and integrity, could more lipid-like surfactants serve the same role? Landau and Rosenbusch proposed this question and came up with a novel way of crystallizing membrane proteins (40, 41). In essence, a preformed surfactant phase with a more membrane-like structure might be used to partition membrane proteins into an environment that would favor close interactions suitable for nucleating and sustaining crystal growth. The bicontinuous cubic surfactant phases made by monoacyl glycerols (16, 42) seem ideal for this purpose as continuous regions of solvent and surfactant extend throughout the phase and can co-exist with a bulk solvent phase. Detergent-solubilized membrane protein, added externally, can easily partition into the bicontinuous cubic phase; the solvent channels allowed the manipulation of the aqueous environment to initiate crystallization. Although many of the assumptions made by Landau and Rosenbusch are not confirmed, their technique allowed the high resolution structure determination of bacteriorhodopsin (43, 44) and halorhodopsin (45).

Lipid Interactions as Observed in Membrane Protein Crystals

The crystal structure of bacteriorhodopsin obtained from the cubic phase system discussed above (43, 44) showed a remarkable feature: a layer of lipid molecules was resolved on the protein surface. The nature of the lipids, originating from the native bacterial membrane, and their positioning in the grooves and crevices of the protein (Fig. 4) suggest specific and well defined protein-lipid interactions. Over the years, numerous studies have demonstrated that membrane lipids are rapidly exchanging at the surface of integral membrane proteins (46), even though a motionally restricted population was observed and quantified by EPR (47). The functional significance of this "annular layer" of lipid has been much debated, but for many purposes the bilayer has been usefully considered as a hydrophobic solvent, albeit complex in its properties (48) (see also the first minireview in this series by White *et al.* (64)). With the advent of high resolution crystal structures of membrane proteins, the observation of protein-bound lipid molecules now appears to be becoming a rule rather than an exception. Moreover, these crystalline complexes of membrane proteins and lipid do not contain just unusual lipids, such as cardiolipin (49) or diether lipids (44), but also more common phospholipids. The structure of bovine cytochrome *c* oxidase at 2.8-Å resolution revealed 5 phosphatidylethanolamine and 3 phosphatidylglycerol molecules per 200-kDa monomer (50). At higher resolution (2.0 Å), 14 phospholipids, including 5 cardiolipin molecules, have been identified,[2] which are still only a subset of the 56 lipids with restricted mobility that have been identified by EPR (47).

These recent crystallographic results imply that lipid may help membrane proteins assume more stable and homogeneous conformations. Hence, many detergents may work best along with retention of some native lipid (51). In contrast, complete lipid removal demands that a detergent must be able to substitute successfully for most, if not all, bound lipid (*e.g.* dodecyl phosphocholine used in NMR structure determination (52, 53)). Nonetheless, the maintenance of some lipid-protein interactions may be critical for procedures like crystallization. The crystal structures of rhodopsin (54) and the sarcoplasmic Ca^{2+} pump (55) emphasize this point. In the case of rhodopsin, minimal purification was used, including a single detergent extraction step (56), whereas the crystallization of the Ca^{2+} pump involved re-addition of lipid (55).

The significance of these findings is profound in terms of how we approach the use of detergents in purification. As mentioned earlier, the complete removal of lipid to obtain monodisperse, homogeneous PDCs was an early goal for x-ray crystallography or NMR

[2] S. Yoshikawa, personal communication.

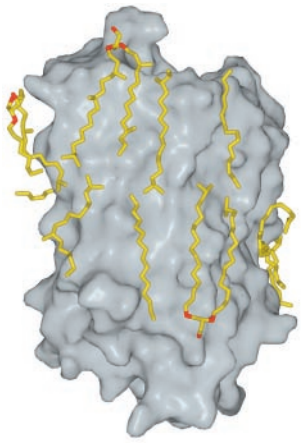

FIG. 4. **A view of membrane protein interactions with lipids.** Native lipids are seen bound to the surface of bacteriorhodopsin in the 1.55-Å crystal structure (44) and suggest intimate and specific interactions between the protein and lipids.

to minimize self-association into insoluble, polydisperse aggregates (28), which is often promoted by phospholipid. However, complete removal of bound lipid from many membrane proteins is rarely achieved and is often detrimental to structure and function (13, 57, 58). Even when reasonably active forms can be maintained in detergent, the structural flexibility/integrity of membrane proteins may be influenced by the loss of associated lipid. For bacteriorhodopsin, NMR studies (59) clearly showed changes as native lipid was removed. Finally, conditions and detergents that can maintain native-like activity (60, 61) may still induce subtle changes that are not detectable in routine assays (57, 62, 63). Hence, complete delipidation may not be the appropriate goal when designing purification procedures with the aim of structure determination (28).

Conclusions

The critical role of detergents in all aspects of membrane protein biochemistry cannot be fully addressed in the context of this short review. As noted above, the behavior of detergents clearly impacts membrane protein purification and crystallization, as well as reconstitution (1), which was not discussed. However, a few generalities can be made that apply to all systems. The nature of the solubilization detergent is an important factor in determining the size and properties of the resulting PDCs. Moreover, the starting lipid content in the purified protein is a critical but often uncontrolled variable. Thus, we come to a new paradigm where "purer is not better" and isolation of specific *protein-lipid* complexes may be the more desirable goal for structural and functional studies of membrane proteins. Banerjee *et al.* (51) showed that different detergents extracted different kinds and amounts of lipids from the same membrane, along with protein, often with significant differences in activity of the isolated protein. Such careful studies may be *de rigueur* for the successful structural analysis of many membrane proteins.

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R. Michael Garavito and Shelagh Ferguson-Miller

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