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Review

The vesicle-to-micelle transition of phosphatidylcholine vesicles induced by nonionic detergents: effects of sodium chloride, sucrose and urea

Anne Walter *, Gwendolyn Kuehl, Kerri Barnes, Gregg VanderWaerdt

Department of Biology, St. Olaf College, 1520 St. Olaf Avenue, Northfield, MN 55057, USA

Abstract

The vesicle-to-micelle transition of egg phosphatidylcholine LUVs induced by octylglucoside was studied in buffers with 0–4 M sodium chloride, sucrose or urea. We used both light scattering and fluorescent probes to follow the lipid–detergent complexes in these buffers. The vesicle-to-micelle transition process was fundamentally the same in each solute. However, the detergent-to-lipid ratio required for micelle formation shifted in ways that depended on the aqueous solute. The partitioning of octylglucoside between the vesicles and the aqueous phase was primarily determined by the change in its critical micelle concentration (cmc) induced by each solute. Specifically, the cmc decreased in high salt and sucrose buffers but increased in high concentrations of urea. Cmc for two additional nonionic detergents, decyl- and dodecyl-maltoside, and three zwittergents (3-12, 3-14 and 3-16) were determined as a function of concentration for each of the solutes. In all cases NaCl and sucrose decreased the solubility of the detergents, whereas urea increased their solubilities. The effects clearly depended on acyl chain length in urea-containing solutions, but this dependence was less clear with increasing NaCl and sucrose concentrations. The contributions of these solutes to solubility and to interfacial interactions in the bilayers, pure and mixed micelles are considered.

Keywords: Critical micelle concentration; Osmotic strength; Maltoside; Octylglucoside; Zwittergent

1. Introduction

Although the vesicle or bilayer-to-micelle transition has been well studied for nonionic detergents and phospholipids, relatively little attention has been given to the effects, if any, of the aqueous phase composition on this transition. The nonionic detergents, such as octylglucoside and dodecylmaltoside, have been considered to be relatively insensitive to the effects of ionic strength since they are uncharged but the effects of solute on the solubility of the hydrocarbon region are expected to be independent of ionic status. Moreover, nonionic solutes such as sucrose and urea may alter headgroup interactions specifically as well as act nonspecifically through increasing the osmotic strength of the solution. Urea is known as a denaturant but is also found at high

Abbreviations: EPC, egg phosphatidylcholine; OG, octylglucoside; D_{aq}, aqueous monomer concentration of detergent in equilibrium with mixed detergent–lipid structures; OG_{aq}, D_{aq} of octylglucoside; cmc, critical micelle concentration(s); cmmc, critical mixed micelle concentration(s); R_e, effective ratio of detergent/lipid in a given mixed lipid structure; R_{SAT}, ratio of OG/EPC at the saturation point; R_{SOL}, ratio of OG/EPC at the solubilization point; NBD-PE, 4-nitrobenzo-2-oxa-1,3-diazole phosphatidylethanolamine; Rho-PE, lissamine rhodamine B sulfonyl phosphatidylethanolamine; FRET, fluorescence resonance energy transfer; F_{NBD}, fluorescence intensity of NBD-PE; DM, decylmaltoside; DoDM, dodecylmaltoside; zwit 3-12, zwittergent 3-12; zwit 3-14, zwittergent 3-14; zwit 3-16, zwittergent 3-16; ANS, β-anilino-1-naphthalenesulfonate

* Corresponding author. Fax: +1-507-646-3968;
E-mail: waltera@stolaf.edu

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concentrations in some biological systems. Moreover, experimental protocols that involve solubilization or procedures in mixed detergent–lipid–protein micelles are often carried out in media that vary widely in composition. In particular, protein crystallization procedures might use high concentrations of salts, sugars or polyethylene glycol to induce crystallization. Isolated solubilized proteins may be stored in protective media with relatively high concentrations of sucrose or mannitol and then be switched to physiological saline for subsequent procedures. Denaturation studies or isolation protocols may require relatively high concentrations of urea or salts. For these reasons, it is of great practical value to know what the effects of various aqueous phase solutes might be on the process of vesicle solubilization by nonionic detergents.

The effect of the aqueous phase composition is also interesting from a theoretical point of view. The propensity for amphiphiles to aggregate forming particular structures is due to a balance of forces that include the energy of placing the hydrophobic regions into the aqueous medium, the aqueous solubility of the headgroups and the positive (H-bonding, van der Waals) or negative (electrostatic repulsion, steric hindrance) interactions among the amphiphiles. The particular shape of a given amphiphile (for example, the length to cross-sectional area ratio) and the aggregate shapes of a mixture of amphiphiles help determine the actual shape of the structures that form [1–3]. In the case of the vesicle bilayer-to-micelle transition with the addition of detergents, the aggregate shape or spontaneous curvature of the population of amphiphiles, phospholipids and detergents is changing from one that has an energy minimum in a bilayer configuration to one that is more stable in a micellar structure. As is known from previous work [4–7], the shapes of the vesicles and micelles also evolve with changing amphiphile composition. In the octylglucoside (OG)/egg phosphatidylcholine (EPC) system examined here, the micelles evolve from elongated worm-like structures to shorter flexible rods to small ellipsoidal micelles as the proportion of detergent increases [4], a transition pattern common to many detergent/lipid systems. Moreover, the specific mixed micellar shapes and stable compositions are known to vary with changes in the concentration of NaCl when ionic detergents are involved [8,9].

The first step in understanding the vesicle-to-micelle transition is to determine the compositions of lipid and detergent at which the vesicular structure begins to destabilize and at which the system is completely composed of mixed micelles. The model we use is the simple three-stage model consisting of a vesicle or bilayer stage, a coexistence region with mixed bilayer and micellar structures and finally a mixed micellar region [10–12]. For the purposes of this study we ignore divisions within each stage such as the point of leakage in the vesicle/bilayer region [13,14].

The vesicle–micelle transitions can be followed by the changes in turbidity associated with the change in aggregate size as vesicles (about 100 nm diameter in these studies) are lost and micelles (about 1–2 nm in diameter for OG/EPC) appear [15,16]. Similar and additional information can be gathered using the change in energy transfer efficiency between a pair of fluorescent energy transfer probes in the bilayer membrane [15]. If the characteristic features of these light scattering and fluorescence resonance energy transfer (FRET) records are similar over a range of lipid concentrations, the process of solubilization may be considered to be the same. As expected, the actual detergent concentration at which a particular feature (e.g., rapid drop in light scattering) occurs will increase as the concentration of lipid increases but in a nonstoichiometric manner. This is due to the fact that the detergent partitions between the lipid and aqueous phases and only the fraction that is associated with lipid increases stoichiometrically with the lipid concentration while the aqueous monomer concentration is constant for a given composition of the lipid–detergent structures.

The partitioning behavior of the detergent relative to the lipid concentration can be described by two parameters: the ratio of detergent to lipid in the structures (also known as the effective ratio, R_e) and the aqueous monomeric detergent concentration D_{aq} [17]. The R_e in the structures at a particular step in the solubilization process is the slope of a line relating the concentration of detergent to the concentration of lipid. The intercept of this line is D_{aq}. D_{aq} reflects the solubility of the detergent in the aqueous

phase and its free energy in the lipid phase. At the point of complete vesicle conversion to micelles, this aqueous concentration is called the critical mixed micellar concentration, or cmmc [15]. These parameters reflect the ensemble composition and are useful as a means of comparison among various amphiphiles and across systematic changes in physical parameters. R_e at the point of saturation when the bilayers are just about to collapse to micelles (R_{SAT}) and at the point of complete solubilization (R_{SOL}) are useful reference compositions that mark the boundaries of the bilayer-micelle co-existence region. Changes in either of these values with changes in the particular detergent or lipid chemistry reflect differences in their relative packing parameters due to alterations at the bilayer or micellar aqueous interface (e.g., [13]). Changes in the aqueous phase concentration at any point reflect a change in the partition coefficient of the detergent, a parameter that depends on both its solubility in the aqueous phase and its solubility in the lipid phase. The aqueous phase solubility can be measured independently by measuring the critical micelle concentration (cmc) of the detergent in the absence of lipid. Changes in D_{aq} at saturation or solubilization that are not due to changes in the cmc reflect changes in the detergent solubility in or binding to the bilayer. In addition to being dependent on the specific amphiphiles in the system, these parameters (R_{SAT}, R_{SOL}, and D_{aq}, cmmc) can also change with physical parameters such as temperature (e.g., [13,18]).

To understand changes in the parameters describing the vesicle–micelle transition, we must separate the effects of solutes on the detergent solubility in water (and thus its partition coefficient between the aqueous and lipidic phases) from effects such as spontaneous curvature of a given mixed assembly that are specific to the vesicle–micelle transition itself. To do this, we determined the vesicle-to-micelle parameters for a variety of conditions and then independently considered the effects of the aqueous solutes (NaCl, sucrose and urea) on the solubility or cmc of the nonionic detergents. Salts and sucrose are known to be agents that decrease solubility (salting out) whereas urea is among a class of solutes known to increase the solubility of hydrophobic compounds [2,19]. Salts are also well known to alter the behavior of ionic detergents through electrostatic, and sometimes specific, binding. The effect of decreasing solubility in water is to increase the partition coefficient between the amphiphile and a lipid structure as observed, for example, for cholate, CHAPS, and both the charged and uncharged forms of tetracaine [9,20]. Urea, on the other hand, increased the solubility of both forms of tetracaine in the aqueous phase and consequently, decreased its lipid/water partition coefficient [20]. Urea is also reported to facilitate solubilization of the saturated PCs by Triton X-100 at temperatures well below their respective phase transition temperatures [21]. Sucrose is well known to decrease solubility of other solutes as its concentration increases in water. However, it is also known to be a protective agent for lipid bilayers under dehydration and freezing conditions [22,23]. Thus, it is likely that these solutes will alter the vesicle–micelle transition but in very different ways.

To separate the cmc change from the changes in the bilayer and micellar structures themselves, the detergent cmc can be determined over the range of conditions of interest (e.g., temperature, salt). The relative importance of headgroup and acyl chain effects on detergent solubility can be examined by systematically altering the detergent structure. Given that the three solutes being tested herein have different effects that are all related to their concentrations, one outcome might be that each of the three solutes will exert an effect on the solutes in direct proportion to their concentrations. If the solute effects are simple colligative properties, they should scale to the osmotic strength of the solution. If these effects are primarily through changes in the solubility of the nonpolar region, these changes should scale with acyl chain length. Differences due to changes in the polar headgroup solubility or effective size are predicted to result in differences among detergents with differing headgroups.

In this report, we have assembled the solubilization data for the egg phosphatidylcholine (EPC)/octylglucoside (OG) system as a function of the aqueous phase solute composition. In particular, we altered NaCl, sucrose and urea concentrations from 0.01 to 2 M in a 10 mM Na–Hepes buffer. We chose the egg PC/OG system because it is extremely well characterized (e.g., [4,7,11,15,16,24–27]). In addition, OG appears to have no specific interactions with PC

and thus mixes ideally in the lipid structures [15,27] The goal of this work was to first discover if and how these solutes altered the vesicle-to-micelle transition. Then, in order to ascertain the source of the solute effects, we measured the critical micelle concentrations of several nonionic and zwitterionic detergents in the same solvents. The data are compared in terms of the solution osmolality in order to compare any differences in the solute effects separately from differences in the water activity of the system.

In brief, all three solutes did alter the detergent/lipid ratios at solubilization, the width of the bilayer-vesicle coexistence region, the aqueous detergent concentrations and the cmc of the detergents, but in different ways. These results suggest that the way in which the solutes interact with water and with the amphiphilic molecules both contribute to the dependencies on aqueous solute composition. However, there were only a few subtle changes in the overall process of solubilization itself. Changes in micelle size and shape were not measured by our techniques but the appearances of some of the samples suggest that such changes did happen. These data have been presented in abstract form [28,29].

2. Materials and methods

2.1. Buffer preparation

Buffers of different concentrations of solute were prepared. All buffers contained 10 mM Hepes, 0.1 mM EDTA (Sigma), 0.02% sodium azide, and the indicated concentrations of NaCl, sucrose and urea (Fisher Chemicals). Sodium azide was used as a preservative to prevent the growth of bacteria in each buffer. The pH was adjusted to 7.2 with NaOH. The osmolality of each of the buffers was measured using a Wescor Vapor Pressure Osmometer Model 5100C (Logan, UT). Osmolalities were also taken from the Handbook of Chemistry and Physics (47th edition).

2.2. Lipid vesicle preparation

Egg phosphatidylcholine (EPC), 4-nitrobenzo-2-oxa-1,3-diazole phosphatidylethanolamine (NBD-PE), and lissamine rhodamine B sulfonyl phosphatidylethanolamine (Rho-PE) were obtained in chloroform from Avanti Polar-Lipids (Alabaster, AL). These were combined in a clear dry round-bottomed tube to create a mixture that was 0.7 mol% NBD-PE and Rho-PE in EPC. The tube was rotated under a stream of nitrogen to evaporate the chloroform and deposit the lipid as a thin film on the side of the tube. One ml of the appropriate buffer was added to hydrate the lipids and the sample was periodically vortexed to create a dispersion of multilamellar vesicles. The solution was frozen with liquid nitrogen, then thawed and vortexed ten times prior to extrusion through 0.1-μm filters (Nucleopore, Pleasanton, CA) with an Extruder (Lipex Biomembranes, Vancouver, Canada). All mixtures were extruded ten times to ensure uniform unilamellar vesicles. We measured the organic phosphate concentration in a small aliquot of our lipids to determine the concentration of phospholipids in the final preparation [30]. When vesicles were prepared at the higher sucrose and urea concentrations, the initial step of the assay, hydrolysis of the lipid phosphate, had to be adjusted by the addition of excess $MgNO_3$ to compensate for the large amount of extra carbon available for oxidation.

2.3. Vesicle fluorescence and light-scattering measurements

In order to follow the changes in vesicle structure and micellization at the molecular level, we measured the change in donor fluorescence and light scattering during continuous detergent addition [15]. The vesicles were prepared with 0.7 mol% each NBD-PE (donor) and Rho-PE (acceptor), conditions that result in efficient energy transfer. We monitored the donor fluorescence at an excitation of 470 nm and emission of 535 nm: emission spectra confirmed that there was a significant acceptor (Rho-PE) fluorescence emission at 590 nm from the intact vesicles which decreased as the F_{NBD} increased. As the detergent was added, the spacing between the probes increased and the energy transfer efficiency decreased. As micelles form, the probability that an NBD-PE and a Rho-PE would be in the same structure decreases [24], and thus, energy transfer decreases significantly as shown by an increase in donor (NBD-PE) fluorescence (Fig. 1).

We followed the vesicle-to-micelle transition structurally by changes in the 90° light scattering (470/470 nm) associated with solubilization and the changes in the size of our structures. Light scattering is a sensitive indicator of changes in the size of the structure as the amount of light scattered depends on the sixth power of the radius of the suspended particles.

An SLM DMX 1000 fluorimeter (Urbana, IL) was used in T-format set to measure fluorescence and scattered light simultaneously. The cuvette holder was water-jacketed at 25°C for all experiments. Initial and final spectra were taken as controls to confirm the status of the vesicles and energy transfer. We measured NBD-PE fluorescence and light scattering continuously as we added 200 mM OG to the EPC vesicles from a syringe pump (Pump 22, Harvard Apparatus, South Natick, MA). By determining each breakpoint at several lipid concentrations, the key parameters (R_e and D_{aq}) were determined at selected points in the process. Each measurement was repeated a minimum of three times.

2.4. Detergents and cmc measurements

Detergent stock solutions were prepared at concentrations of 200 mM octylglucoside (OG), 10 mM decylmaltoside (DM), 1 mM dodecylmaltoside (DoDM), 20 mM zwittergent 3-12 (zwit 3-12), 10 mM zwittergent 3-14 (zwit 3-14), and 1 mM zwittergent 3-16 (zwit 3-16). Their concentrations were checked by comparison with published cmc values in 150 mM NaCl buffer at pH 7.2. The detergents were purchased from Calbiochem (La Jolla, CA).

The critical micelle concentrations of OG, DM, DoDM, and the three zwittergents, zwit 3-12, 3-14 and 3-16, were determined by measuring the fluorescence intensity of β-anilino-1-naphthalenesulfonate (ANS) during continuous detergent addition. The cuvette contained approximately 0.2 mM ANS in 2.2 ml of each buffer and the detergent was added at a rate of 5 or 10 μl/min from the syringe pump. ANS exhibits virtually no fluorescence in water but has a significant fluorescence yield ($\lambda_{ex} = 380$ nm, $\lambda_{em} = 420$ nm) in hydrophobic environments. Thus, an increase in fluorescence represents the availability of micelles into which ANS partitions. The cmc was determined for each condition a minimum of three times.

3. Results

This study of the aggregation and stabilization of vesicles in an aqueous environment was done in two parts. The vesicle-to-micelle transition of EPC induced by OG was determined as a function of concentration of three aqueous solutes: sodium chloride, sucrose and urea. Next, the cmc of several nonionic and zwitterionic detergents were measured under the same conditions. From the changes in solubilization of the OG–PC system and the cmc effects, we can begin to describe the source of the effects on these nonionic amphiphilic systems.

3.1. The effect of solute on the vesicle-to-micelle transition of EPC induced by octylglucoside

EPC vesicles prepared in NaCl, sucrose and urea had similar initial characteristics regardless of the particular aqueous solutes or their respective concentrations. Similarly, the overall transition pathway appeared similar when it was measured by FRET or by changes in light scattering (Fig. 1). In this example, the F_{NBD} increase from 0 to about 17 mM OG due to the separation of the lipid headgroups with the incorporation of OG in to the bilayers until they were

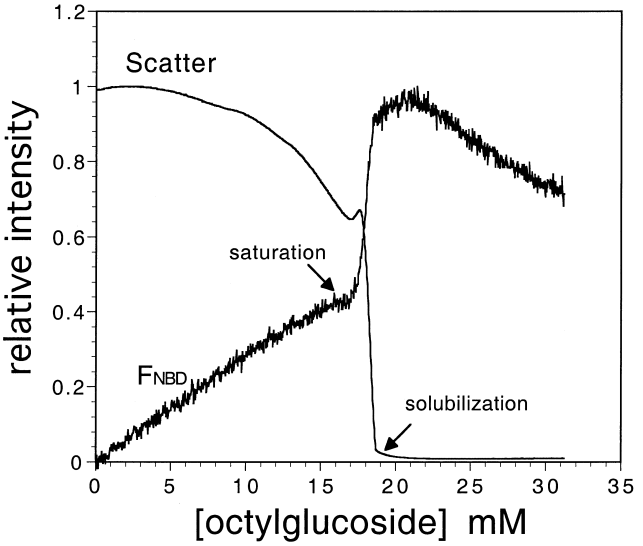

Fig. 1. EPC vesicles solubilized by continuous addition of octylglucoside. F_{NBD} increased with decreasing FRET as detergent was added and the amount of scattered light decreased. The points of onset (saturation) and completion (solubilization) of the transition to mixed micelles are indicated by the arrows.

saturated with OG. The sharp rise in F_{NBD} spans the bilayer-micelle co-existence region. After the fluorescence hits a maximum, there is a drop due to dilution. The scatter curve drops slowly up to the point where micelle formation is initiated i.e., when the drop in scatter is precipitous. This decrease in scattered light represents rearrangement of the large vesicles to smaller structures [4]. The sharp peak just prior to clarity reflects a narrow region in which the system separates to a two-phase system, a detergent rich viscous phase and a lipid rich turbid phase [15]. Although these overall changes were fairly similar in all conditions, the three solutes did alter the OG/PC ratio at the phase boundaries (the potency of the detergent), D_{aq} at saturation and the OG cmmc.

Four parameters were routinely monitored: the ratio of OG/PC at the initiation of micelle formation (R_{SAT}) and at the completion of micellization (R_{SOL}) as well as the OG_{aq} at saturation and the cmmc. As is noted above, these values were determined by plotting the OG concentration at the breakpoints indicated in Fig. 1, against at least five lipid concentrations. The slope of these linear plots is R_e and the intercept is D_{aq}. In 10 mM Na–Hepes with no added solute, R_{SAT} values averaged from several independent data sets were 1.55 ± 0.13 with $[OG]_{aq}$ equal to 16.7 ± 0.44 mM. The average R_{SOL} was 2.62 ± 0.23 with a corresponding cmmc of 17.8 ± 0.25 mM.

3.1.1. Sodium chloride

As the concentration of sodium chloride increased, the overall transition from vesicles to micelles occurred at lower [OG] (Fig. 2A). The scatter curves were quite similar in shape with one exception: the small peak in the scatter curve representing the narrow phase separation region became increasingly sharp with increased NaCl. The highest salt concentrations also seemed to have a tiny second peak just after complete micellization: this has not been characterized.

From data obtained at several lipid concentrations, we determined R_{SAT} and R_{SOL} at each [NaCl]. R_{SAT} increased from 1.41 at low ionic strength to 1.77 in 1.5 M NaCl, suggesting that the amount of detergent needed to induce micelle formation (or that could be tolerated in a bilayer structure) increased somewhat (Table 1). Similarly, the value for R_{SOL} increased from 2.36 in 0 M NaCl to 3.35 in 1.5 M NaCl. The width of the transition region increased meaning that bilayer saturated with detergent coexisted with mixed micelles over a wider range of R_e or OG/PC ratios. OG_{aq} decreased from 17 mM at saturation in 0 NaCl to 7.52 mM at 1.5 M NaCl. Similarly, the cmmc decreased from 17.8 mM to 7.71 mM in high salt (Table 1 and Fig. 3A). The magnitude of this change relative to the increase in NaCl is similar to, but less than, the change in the cmc of OG as the concentration of NaCl increases (Fig. 3A). In both cases, the effect was nonlinear with the osmo-

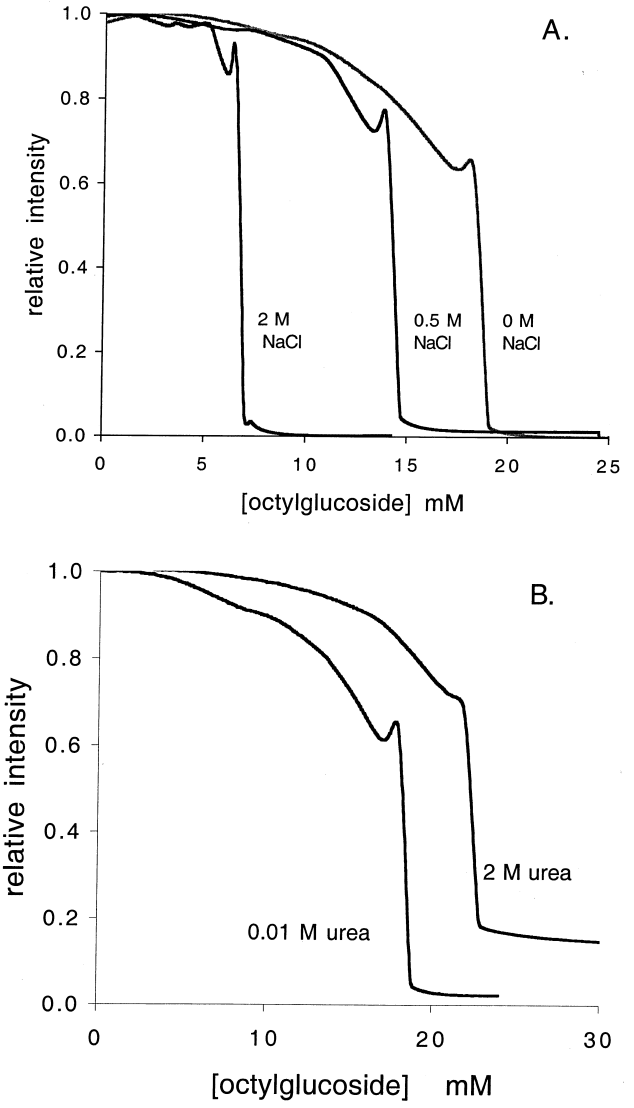

Fig. 2. EPC vesicles solubilized by continuous addition of octylglucoside in (A) 0, 0.5 and 2 M NaCl and (B) 10 mM and 2 M urea. In this example, solubilization was followed by the change in scattered light (470/470 nm).

Table 1
NaCl effects on the vesicle-to-micelle transition parameters for the EPC/OG system

[NaCl] (mM)	R_{SAT}	$[OG]_{SAT}$ (mM)	R_{SOL}	$[OG]_{SOL}$ (mM)
0	1.41	17.0	2.36	17.8
20	1.75	16.8	2.96	17.3
100	1.69	16.1	3.12	16.8
500	1.82	12.8	3.19	13.2
1000	1.75	9.83	3.29	10.0
1500	1.77	7.52	3.35	7.71
2000	–	–	3.39	5.95

At least five EPC concentrations were examined to determine these values.

lality of the NaCl solutions becoming less steep at the higher concentrations.

3.1.2. Sucrose

In sucrose, the total concentration of OG needed for micelle formation decreased with increasing sucrose concentrations. However, the increases in R_{SAT} and R_{SOL} with increasing sucrose concentration were greater than they were for sodium chloride, reaching 2.31 and 4.29 for R_{SAT} and R_{SOL}, respectively, at 2 M sucrose (Table 2). This suggests that sucrose stabilized bilayer structures. The OG_{aq} at these two points dropped to 9.3 and 10.1 mM, respectively, in comparison with the OG cmc of 11.8 mM in 2 M sucrose (Fig. 3B) representing decreases to 55%, 58% and 53% of their respective 0 sucrose values.

3.1.3. Urea

In contrast to NaCl and sucrose, increasing the concentration of urea resulted in higher total OG concentrations at solubilization (Fig. 2B and Table 3). Again, the shapes of the scatter curves were similar in low and high urea but the phase separation peak was less prominent in high urea. When the osmolality of the urea solutions increased from 0 to 4.37 OsM (4 M urea), the aqueous concentrations of OG rose from 16.2 to 23.7 mM and from 18.0 to 25.5 mM, respectively, at saturation and solubilization (Table 3 and Fig. 3C). R_{SAT} and R_{SOL} were 1.63 and 1.8 at 4.37 OsM or 4 M urea, similar to, and significantly lower than their respective values at 0 urea, respectively (Table 3). In this case the structural composition width of the transition decreased with increasing urea so that in 4 M urea, the coexistence region was extremely narrow. When we examined the light-scattering curves for urea (Fig. 2B), we noted that the small peak that occurs just prior to solubilization is much less prominent than in the low urea solution. The light scattering due to the mixed

Table 2
Sucrose effects on the vesicle-to-micelle transition parameters for the EPC/OG system

[Sucrose] (mM)	R_{SAT}	$[OG]_{SAT}$ (mM)	R_{SOL}	$[OG]_{SOL}$ (mM)
0	1.66	16.9	2.79	17.5
10	1.78	16.6	2.97	17.2
500	1.53	15.1	2.70	15.7
2000	2.31	9.3	4.29	10.1

At least five EPC concentrations were examined to determine these values.

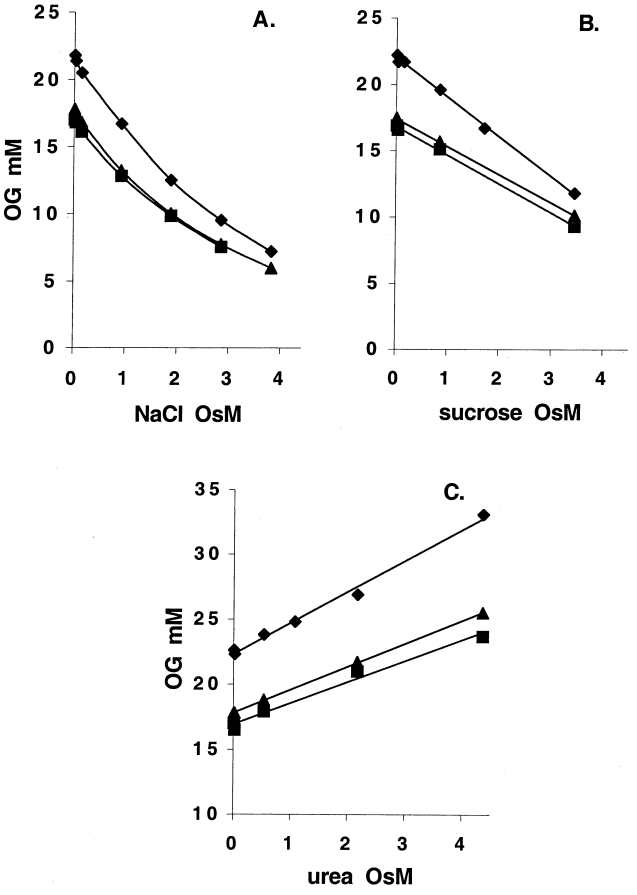

Fig. 3. The cmc (♦), the cmmc (■) and the values of OG_{aq} at saturation (▲) are shown as a function of the osmolality of NaCl (A), sucrose (B) and urea (C). For sucrose and urea the relationship appears to be a straight line as indicated by the linear regression fit presented on the graph.

Table 3
Urea effects on the vesicle-to-micelle transition parameters for the EPC/OG system

[Urea] (mM)	R_{SAT}	$[OG]_{SAT}$ (mM)	R_{SOL}	$[OG]_{SOL}$ (mM)
10	1.53	16.4	2.7	18.0
500	1.46	17.7	2.8	18.8
2000	1.29	21.0	2.5	21.8
4000	1.63	23.7	1.8	25.5

At least five EPC concentrations were examined to determine these values.

micelles at solubilization was greater in high urea relative to low urea concentrations suggesting that these micellar structures themselves might be larger.

3.2. The effects of sodium chloride, sucrose and urea on detergent cmc

3.2.1. Octylglucoside

As noted above, all three solutes altered the cmc of OG as the solution osmolalities were increased. For example, the effect of increasing NaCl concentration can be seen in the shift to the left of the rise in ANS fluorescence during continuous OG addition to well-stirred cuvettes containing 0 to 2 M NaCl (Fig. 4). The OG cmc decreased as concentrations of NaCl and sucrose increased and increased with increasing urea concentrations (Tables 4–6). When the OG cmc were compared with the OG/EPC cmmc, we saw, as has been shown before (e.g., [15,31]), that the cmc are higher and that there is a small difference in the OG_{aq} at saturation and solubilization. In all cases the cmc of OG were altered to a slightly greater

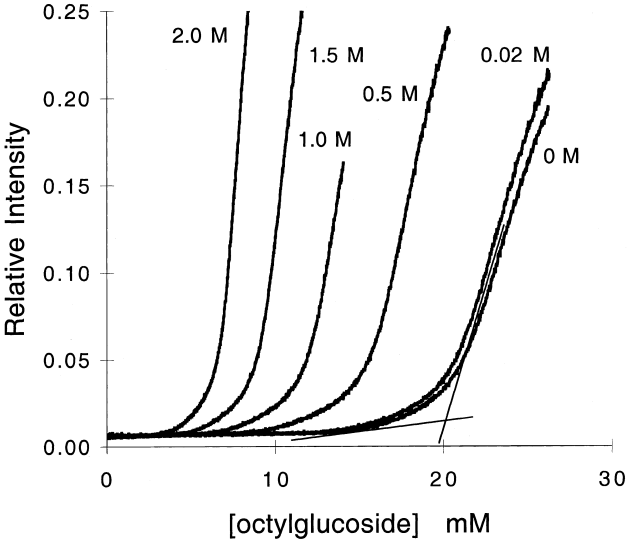

Fig. 4. Critical micelle concentrations (cmc) were determined using the fluorescent probe ANS. This example is of the cmc of OG in buffer solutions varying in NaCl from 0 to 2 M. The concentration of OG at which a sharp increase was observed in the fluorescence intensity (indicated for 0 NaCl on the graph) was defined as the cmc.

extent than OG_{aq} in equilibrium with mixed lipid–detergent structures (Fig. 3A–C). From this we can infer that each of the solutes also perturbed the free energy of the structures themselves consistent with the changes in R_{SAT} and R_{SOL} described above.

3.2.2. The cmc of the maltosides and the zwittergents in NaCl, sucrose and urea solutions

To explore further the basis for the effects of these solutes on detergent solubility, we determined the cmc of DM, DoDM, and zwit 3-12, 3-14 and 3-16

Table 4
Detergent cmc in NaCl

[NaCl] (M)	OsM (mol/kg)	OG cmc (mM)	DM cmc (mM)	DoDM cmc (mM)	Zwit 3-12 cmc (mM)	Zwit 3-14 cmc (mM)	Zwit 3-16 cmc (mM)
0	–	21.8	1.69	0.17	1.82	12.1×10^{-2}	11.3×10^{-3}
0.2	0.24	21.4	1.60	0.17	–	–	–
0.5	0.92	16.7	1.71	0.12	1.26	8.86×10^{-2}	9.0×10^{-3}
1.0	1.89	12.5	1.17	0.10	–	6.32×10^{-2}	6.3×10^{-3}
1.5	2.86	9.5	0.90	0.07	–	3.85×10^{-2}	4.1×10^{-3}
2.0	3.83	7.2	0.57	0.04	0.37	2.68×10^{-2}	3.1×10^{-3}

The critical micelle concentrations of octylglucoside (OG), decylmaltoside (DM), dodecylmaltoside (DoDM) and zwittergent 3-12, 3-14 and 3-16 in NaCl solutions ranging from 0 to 2 M in NaCl. Each solution was buffered to pH 7.2 with 10 mM Na–Hepes, and contained 0.1 mM EDTA and 0.02% sodium azide.

Table 5
Detergent cmc in sucrose

[Sucrose] M	OsM (mol/kg)	OG cmc (mM)	DM cmc (mM)	DoM cmc (mM)	Zwit 3-12 cmc (mM)	Zwit 3-14 cmc (mM)	Zwit 3-16 cmc (mM)
0	–	22.2	1.91	0.18	1.93	0.147	1.13×10^{-2}
0.01	–	21.7	1.89	0.17	1.94	–	–
0.1	0.15	21.7	1.87	0.17	–	–	–
0.5	0.85	19.6	1.71	0.16	1.84	0.133	1.10×10^{-2}
1.0	1.72	16.7	1.55	0.15	1.68	0.131	1.08×10^{-2}
2.0	3.45	11.8	0.87	0.10	1.24	0.106	–

The critical micelle concentrations of octylglucoside (OG), decylmaltoside (DM), dodecylmaltoside (DoDM) and zwittergent 3-12, 3-14 and 3-16 in sucrose solutions ranging from 0 to 2 M in sucrose. Each solution was buffered to pH 7.2 with 10 mM Na–Hepes, and contained 0.1 mM EDTA and 0.02% sodium azide.

in the three different solutes. These are all commonly used detergents that represent two different headgroups, the nonionic maltoside and the zwitterionic head group of the zwittergents. We also varied the length of the hydrocarbon tails using C10 and C12 maltosides and C12 to C16 zwittergents.

As the concentration of NaCl increased, the cmc of all of the detergents examined decreased (Table 4). One way to compare the relative magnitude of the effect of salt on each is to express the cmc at a given solute concentration as a percentage of the cmc in the absence of salt. For example, at 2 M NaCl the cmc are 34% and 24% of the value at 0 salt for DM and DoDM. The cmc of the zwittergents decreased as well, with the cmc in 2 M NaCl for zwit 3-12 being 20% of the cmc in 10 mM Hepes buffer, and those of for zwit 3-14 and 3-16 being 22% and 27%, respectively. The effect of NaCl on both the maltosides and the zwittergents showed dependence on chain length but in the opposite directions. The maltosides were affected more with increasing chain length and the zwittergents experienced a slightly smaller change due to high salt as the chain length of the tail increased.

Similarly, the cmc decreased with increasing concentrations of sucrose in the medium. In 2 M sucrose, the cmc of DM and DoDM were 46% and 56% of their respective cmc in Hepes buffer with no sucrose (Table 5). Unlike the effect of sodium chloride on the maltosides, the effect of increasing sucrose concentrations was less as the detergent chain length increased from C10 to C12, i.e., DM to DoDM. The zwittergent series also was progressively less affected by sucrose as the chain length increased with the cmc in 2 M sucrose being 64% and 72% of the 0 sucrose values for zwit 3-12, -3-14, with virtually no change in the cmc of zwit-3-16 with added sucrose. The maltoside headgroup may be an important factor in this case as OG with its smaller headgroup was less affected than DM

Table 6
Detergent cmc in urea

[Urea] (M)	OsM (mol/kg)	OG cmc (mM)	DM cmc (mM)	DoDM cmc (mM)	Zwit 3-12 cmc (mM)	Zwit 3-14 cmc (mM)	Zwit 3-16 cmc (mM)
0	–	21.8	1.9	0.18	1.95	0.121	1.13×10^{-2}
0.01	–	22.3	1.93	0.17	1.96	–	–
0.5	0.53	23.8	2.1	0.19	2.21	0.165	1.50×10^{-2}
1.0	1.08	24.8	2.32	0.22	2.61	0.195	1.61×10^{-2}
2.0	2.18	26.9	2.69	0.28	3.23	0.279	2.68×10^{-2}
3.0	3.27	–	–	–	–	0.426	3.76×10^{-2}
4.0	4.37	33.1	3.54	0.41	–	–	–

The critical micelle concentrations of octylglucoside (OG), decylmaltoside (DM), dodecylmaltoside (DoDM) and zwittergent 3-12, 3-14 and 3-16 in urea solutions ranging from 0 to 4.0 M. Each solution was buffered to pH 7.2 with 10 mM Na–Hepes, and contained 0.1 mM EDTA and 0.02% sodium azide.

(53% change in OG's cmc) and zwit 3-12 was certainly less affected by sucrose than DoDM despite their identical hydrocarbon tails.

In urea, the cmc for these three detergents increased as was noted above for OG (Table 6). At 2 M urea, the cmc were 123%, 142%, and 156% of their 0 urea values for OG, DM and DoDM. For the zwittergents the effect was 166%, 231% and 237% for the 12, 14 and 16 carbon chains. Thus for urea, the effect increased with the increase in chain length for both the nonionic detergents and for the zwittergents.

3.3. Comparison among the detergent cmc in the three types of aqueous solution

In order to compare the solute effects on each of the detergents, we plotted the log cmc as a function of the solution osmolality as is shown for urea (Fig. 5). By taking the log of the cmc we made it possible to compare each detergent despite differences of several orders of magnitude in their respective cmc values. Since the relationships were linear, it was possible to fit them by linear regression. The slopes of these lines represent the magnitude of the effect on the detergent cmc of each solute with a steeper dependence on solute osmolality being equivalent to a greater dependence. It is possible to compare what the cmc would be at a constant osmolality using these linear relationships. For example, at 1 OsM the OG cmc is predicted to be 12.5 mM in NaCl, 17.5 mM in sucrose and 24.8 mM in urea.

A summary of these results is presented in Fig. 6 where the slopes relating the log of the cmc and the solution osmolality are compared for each detergent in NaCl, sucrose and urea. A few observations are clear. First, the magnitude of the effect of NaCl as a

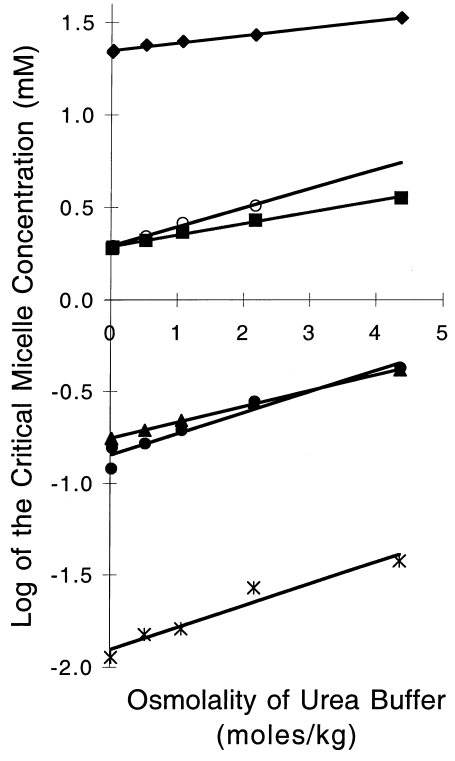

Fig. 5. To compare the effect of osmolality, the log of the cmc of OG (♦), zwit 3-12 (○), DM (■), DoDM (▲), zwit 3-14 (●) and zwit 3-16 (×) are plotted as a function of the osmolality of urea buffers. The lines represent the linear regression for this relationship. The slopes of these lines ($\Delta\{\log(\text{cmc})\}/\Delta\pi_{urea}$) and the equivalent lines for the NaCl and sucrose experiments are compared in Fig. 6.

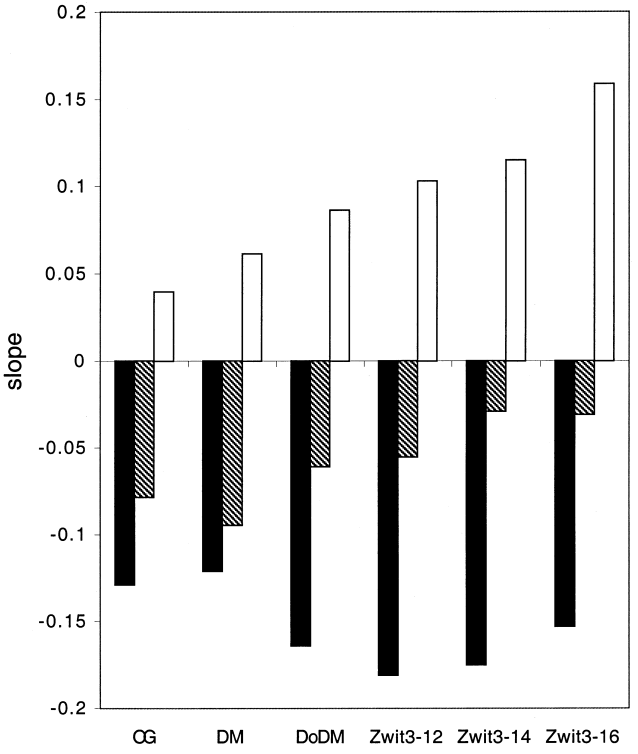

Fig. 6. A summary of the effects of the solutes' osmolalities on the cmc of the six detergents tested is presented. The slopes of the log (cmc) as a function of osmolality are from plots such as those shown in Fig. 5. The solid bars are for cmc determined as a function of NaCl osmolality, the striped bars are the sucrose data and the open bars are the urea values for the six detergents indicated.

function of osmolality was greater than that of sucrose although both decreased the solubility of these detergents. Urea was the only solute to increase detergent solubility and the only solute that appeared to alter cmc in a way that correlated with acyl chain length for both families of detergents. Sucrose effects also tended to be related to chain length but became less strong with increasing chain length. Specifically, the cmc of DoDM depended less on the osmolality of sucrose than did the cmc of DM and the cmc of zwit 3-14 and 3-16 was less affected by sucrose osmolality than the cmc of zwit 3-12. The NaCl osmolality had a greater effect on the cmc of DM than DoDM. However, the steepness of the NaCl effect was progressively less for the zwittergents with the increase in chain length from C12 to C16 although the chain length dependence was not very strong. In fact, the NaCl data suggest competing effects on the headgroup and acyl chain solubilities.

To determine if the solutes contributed to changes in the headgroup solubility, the cmc of the single pair of detergents with identical chain length (DoDM and zwit 3-12) were compared. The cmc of zwit 3-12 was more dependent on NaCl concentration than was the cmc of DoDM. In sucrose, the cmc dependence on sucrose concentration was almost identical for DoDM and zwit 3-12, whereas, the dependence of zwit 3-12 on urea concentration was slightly greater than that of DoDM.

4. Discussion

As expected, increasing the osmolality of NaCl, sucrose or urea in the buffer affects the solubility of detergents in the aqueous phase, which in turn alters the parameters associated with the vesicle–micelle transition. With the possible exception of NaCl, the effects of these aqueous solutes were colligative, i.e., directly proportional to their solution osmolality (e.g., Figs. 3 and 6). However, the amplitudes of each solute's effects on solubility and on the values of R_e were different (Fig. 3 and Tables 1–3). Comparisons among the cmc measurements indicate that these effects are not limited to the solubility of the nonpolar regions of the molecules and may shed some light on the factors determining the aqueous solubility of hydrocarbons (Fig. 6 and Tables 4–6).

4.1. Aqueous solute effects on R_{SAT} and R_{SOL}

Although the change in OG solubility (i.e., its cmc) is clearly the more important change in terms of the total amounts of OG needed to reach a particular stage in the vesicle-to-micelle transition, the changes in R_{SAT} and R_{SOL} are large enough to be interesting theoretically and significant practically. Moreover, if the changes in R_{SAT} and R_{SOL} observed for OG are indicative of what will occur with lower cmc detergents such as DoDM, then the R_e changes will dominate the equation defining the total amount of detergent needed to achieve a certain state in the vesicle–micelle transition.

These parameters (R_{SAT} and R_{SOL}) reflect the stability of the detergent-saturated bilayer and lipid-saturated mixed micellar structures. Increasing NaCl increased both values (by 26% and 42%, respectively) and increased the width of the co-existence region (Table 1). The effect of sucrose is the same but significantly greater both in terms of the changes in R_{SAT} and R_{SOL} and in the width of the coexistence region (Table 2). In contrast, urea lowers R_{SOL} and narrows the co-existence region such that in 4 M urea the transition from bilayer to mixed micelles occurs over a very narrow concentration range with almost no region of co-existence (Table 3). What do we know about these solutes that might explain these differences?

The effects of NaCl are twofold. First, it is an osmoticant that lowers the activity of water and thus increases the activity of all other solutes in the aqueous medium [19]. Secondly, NaCl is a salt that provides significant electrostatic shielding as its concentration increases. At high concentrations, Na^+ binds to the phosphate headgroup of PC [32]. Thus, one reason for greater stability of the bilayer in the presence of OG and the necessity of more OG/PC to form mixed micelles, might be electrostatic shielding. Although the PC headgroups are zwitterionic, the charges act independently. Thus, shielding these charges would permit closer headgroup packing effectively changing the shape of the lipid in a way that decreases the spontaneous radius of curvature [9]. Conversely, the effect may be indirect due to dehydration at the surface decreasing the solubility of the choline headgroup. To discriminate among these possibilities, further experimentation is needed

with charged lipids such as phosphatidylserine and other salts (e.g., Na_2SO_4 to permit higher ionic strength at low osmotic strength).

Sucrose also widens the transition region and increases both R_{SAT} and R_{SOL}. Sucrose is known to be protective for phospholipid vesicles and other membrane systems under dehydration and cryogenic conditions [22,23,33]. One reason sucrose is thought to exert cryoprotective effects, is that its –OH groups can form hydrogen bonds that substitute for those formed between water and the phospholipid headgroups. If this is true under normal hydration states, then sucrose should effectively stabilize the bilayer structure by maintaining hydration and the planar surface in favor of the more highly curved surfaces of the micelles. Other sugars and sugar alcohols such as trehelose and mannitol are known to have quantitatively different degrees of association with phospholipid headgroups and different cryoprotective and dehydration protection potencies. Thus, these solutes are candidates for further exploration designed to unravel the sucrose effects reported here.

In contrast, urea has almost no effect on R_{SAT} but does lower R_{SOL} narrowing the composition of the transition region. Unlike sucrose, urea does not bind to the phospholipid headgroups [33]. As the presence of urea increases the solubility of nonpolar compounds in water [2,34], we infer that urea in the aqueous phase decreases the energetic cost of water penetration into the hydrophobic region of the bilayer. This would make it easier for the bilayer to bend consequently exposing -CH_2-groups to the aqueous phase and achieving the high radius of curvature commensurate with a micellar structure. The narrow width of the transition region suggests that the R_{SAT} structure is relatively unstable and readily converts to a high radius of curvature micellar structure.

4.2. The solubility of detergents in salt, sucrose and urea aqueous solutions

Although the changes in solubilities observed correlate well with the activity of water, there are clearly specific solute effects in both the magnitude and direction of changes observed in the detergent cmc. These suggest that there are both acyl chain and headgroup effects. Salting-out effects are well known [2,19]. Thus, one would expect that increasing the osmolality of the aqueous solution would decrease the solubility of both the headgroup and the hydrocarbon tail and consequently decrease the cmc of a detergent. On the other hand, if the observations were primarily through alterations in the 'hydrophobic effect', the change should be greater with increasing acyl chain length. Although we observe some trends that suggest this to be the case, there are enough exceptions to underscore that there are differences among the solutes and how they interact with the detergent and detergent–lipid system. To separate polar headgroup solubility changes from those of the acyl chain, we used two different types of headgroups and a series of different acyl chain lengths. In NaCl, a 12-carbon acyl chain with the zwittergent headgroup is more sensitive to NaCl concentration than is the corresponding nonionic maltoside, DoDM.

The data in urea solutions are particularly interesting. First, the urea effects are very dependent on detergent acyl chain length (Fig. 6). Second, OG seems to fit in the DM and DoDM series and there is only a small difference between the cmc dependence on urea osmolality for the nonionic DoDM and zwit 3-12. Together, these data imply that most of the effect is on the solubility of the hydrocarbon region and the effect is to increase this solubility. Urea has six possible hydrogen-bonding opportunities and can therefore, disrupt the water–water hydrogen bound lattice by substitution. One hypothesis is that this structure-breaking activity increases the degrees of freedom (entropy) available to water and breaks or relaxes the 'ice-like' water structure thought to occur when a nonpolar solute (e.g., an acyl chain) is placed in water. Exactly how urea's presence in an aqueous solution increases the solubility of nonpolar molecules has been examined for years (e.g., [2,34–36]. The effect cannot be explained exclusively by changes in the water structure itself and the most likely explanation is that urea participates in the solubilization of nonpolar compounds [36]. One experimental approach to resolving the question might come from biological systems that must withstand the effects of high urea concentrations. Urea is a potent DNA and protein denaturant as it is able to stabilize relatively unfolded proteins in solution relative to the folded state. In natural systems with high urea concentrations (e.g., cartilagi-

nous fish, renal medulla), the effects of urea appear to be counteracted by osmolytes such as betaine and other glycine-based compounds [37]. To determine the extent to which this relationship between urea and betaine in aqueous solution can be generalized, it might be interesting to pursue the specific effects of these mixtures to further understand the roles of each solute in mixed amphiphile systems.

In summary, this brief exploration into the effects of the aqueous phase on the behavior of nonionic and zwitterionic amphiphiles clearly indicates that salts, sugars and urea or urea-like compounds such as guanidinium cannot be ignored. These experiments each suggest additional experimental approaches noted above that might aid in understanding the balance of forces that determine solubility, micellization, micelle structure, and the more complex behavior of mixed systems that can be in bilayer and micellar configurations. These, in turn, may aid in understanding the determinants of particular pathways for solubilization, phase separation and vesicle formation.

4.3. Practical significance

Finally, the results presented here are of significant practical value. The effects of salt, sucrose and urea on the solubilization of vesicles and on the solubility of a range of detergents are sufficiently significant to be a consideration when developing a reconstitution protocol, carrying out protein purification or developing crystallization procedures. The most successful protein reconstitution protocols are those that just barely solubilize the protein in order to maintain a high proportion of lipid in the mixed micelle and then quickly return the protein to a bilayer environment. Here we show that switching buffers from salt to sucrose to 'protect' glycosylated proteins or using a salt gradient to elute a protein from a column, is likely to change the state of the protein's lipid environment. As we have shown previously with temperature [18], it is certainly feasible, however, to use these effects advantageously to rapidly change the state of a mixed system between micelles and saturated bilayers. This is often of value in a reconstitution protocol designed to avoid a long exposure to the transition region in which many proteins are subject to aggregation. Thus, by considering carefully buffers and protocols it is possible to avoid difficulties and, perhaps, use to advantage, the effects of the aqueous phase on the vesicle-to-micelle transition.

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