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Effects of glycerol and urea on micellization, membrane partitioning and solubilization by a non-ionic surfactant

Hiren Patel, Gaurav Raval, Mozhgan Nazari, Heiko Heerklotz *

Leslie Dan Faculty of Pharmacy, University of Toronto, 144 College st, Toronto ON, Canada, M5S 3M2

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ABSTRACT

We have studied the effect of two cosolvents, urea and glycerol, on the association and interactions of a surfactant, octaethyleneglycol dodecyl ether ($C_{12}EO_8$) and a phospholipid (POPC). We have measured the CMC, the partition coefficient, the effective mole fractions X_e^{sat} and X_e^{sol} at the onset and completion of the membrane-to-micelle transition (membrane solubilization), and the enthalpies of transfer of surfactant by ITC. Changes in membrane order and dynamics were characterized by time-resolved fluorescence anisotropy measurements of DPH, and micelle sizes and clouding by light scattering. The cosolvents have complex effects that are not governed by the well-known 'salting in' or 'salting out' effects on the solubility alone. Instead, urea and glycerol alter also the intrinsic curvature ('effective molecular shape') of the detergent and the order and packing of the membrane. These curvature effects have an unusual enthalpy/entropy balance and are not additive for lipid and detergent. The results shed light on the phenomena governing lipid–detergent systems in general and have a number of implications for the use of cosolvents in membrane protein studies.

1. Introduction

The study of membrane proteins has become one of the key topics of biomedical research, and virtually all of these investigations require the use of detergents for the isolation of the protein from lipid membranes and its subsequent purification, characterization or reconstitution. Whereas the physico-chemical basics of lipid–detergent interactions in simple, binary systems are quite well understood (for reviews, see [1,2] and papers in Alonso and Goñi's special issue [3]), practical applications often require complex mixtures of detergents and cosolutes. In many cases, there are no suitable models to treat these multi-component systems and therefore, the technical procedures are developed and optimized on a largely empirical level. One example is Grisshammer's magical "triple detergent buffer" [4,5] which allows for the isolation and study of several G-protein coupled receptors (see also [6–8]) and comprises CHAPS, lauryl maltoside, cholesteryl hemisuccinate, 30 v% of glycerol, Tris, and NaCl. The success of this sophisticated system raises the questions why so many components are needed and what their functions are and their effects on protein, lipids, and co-solutes, respectively. We have started to tackle this problem by deriving and validating a model that describes the additive action of two or more detergents, such as CHAPS/LM, to solubilize a membrane [9]. Here we address the effect of glycerol and, for comparison, urea, on the self association, membrane partitioning, and membrane solubilization of non-ionic detergents. Further to its application upon membrane solubilization, glycerol is widely used to stabilize the native structure of globular proteins and to protect cells upon drying and freezing (including technical procedures as well as biological systems such as overwintering insects, etc.).

The effect of glycerol to stabilize the compact, native structure of globular proteins has been explained by several models, describing it as kosmotropic (a water-structure maker) and, according to Timasheff's concept [10,11], as preferentially excluded from the protein surface. This concept can be considered an application of Gibbs' adsorption isotherm (a macroscopic view of solute-induced changes in interfacial tension) at the molecular level: The minimization of free energy causes a solute that would increase the interfacial tension between two media (energetically unfavorable) to be preferentially excluded from this interface. This is the least of evils but the interfacial tension is, nevertheless, increasing due to the loss of mixing entropy in the interfacial region so that the system tends to minimize the interfacial area (e.g., of a protein by assuming a more compact state). MD simulations have provided detailed insight into the effect of glycerol on the composition, relaxation kinetics, and H-bonding of the solvation shell of a protein [12]. Specific details and extent of the interfacial effects depend on the molecular properties of the surfaces.

On a lipid membrane surface, the degree of exclusion of glycerol is even larger than adjacent to proteins [13]. This increases the interfacial tension of the membrane and favors structures with condensed interface. Koynova et al.'s [14] detailed model quantifies the resulting tendencies to freeze of a fluid bilayer into a gel phase [13,15] or convert it into an inverse hexagonal phase [15–17]. Based

* Corresponding author. Tel.: +1 416 978 1188; fax: +1 416 978 8511.
E-mail address: heiko.heerklotz@utoronto.ca (H. Heerklotz).

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on the increased energy expense of exposing hydrophobic surface to water, one might expect glycerol also to promote the aggregation of a surfactant to micelles (i.e., a lowering of the CMC) or into a separate phase (i.e., a lower cloud point). While the prediction regarding the cloud point depression by glycerol was experimentally validated for $C_{12}EO_8$ [18], the CMCs of $C_{12}EO_8$ [18], Brij 58 (another EO-detergent), and CTAB [19] were found to increase upon addition of glycerol. This appears to indicate an anomaly of the EO-head group and/or glycerol; other kosmotropic cosolvents such as NaCl and sucrose decrease the CMCs of a series of surfactants as expected [20]. However, the fact that CMC-shifts do not scale with chain length also in these examples reveals a substantial impact of glycerol-head group interactions on the CMC. Particularly in case of EO-detergents, this seems to overcompensate the "salting out" of the chains. Controversial seems to be the issue whether the dehydration of the EO head group by glycerol renders the intrinsic curvature of a detergent less positive (thus promoting a lower interfacial curvature by a micellar growth to interconnected structures) [18] or, due to a collapse of the EO chain at the interface, more positive (causing the micelles to shrink) [19].

When it comes to detergent–lipid interactions, other kosmotropic additives like sucrose and NaCl increase the amount of octyl glucoside needed for complete membrane solubilization, which is quantified by the mole fraction X_e^{sol} (i.e., they reduce the capacity of its micelles to solubilize lipid). However, their effect on the onset of solubilization (X_e^{sat}) is weak and apparently complex [20]. No information seems available about the effect of glycerol on lipid–detergent interactions (including solubilization).

We compare the effects of glycerol on lipid–detergent systems with those of urea, a cosolvent that behaves just opposite to glycerol in most respects. Urea is a particularly prominent stabilizer of water-exposed surfaces that accumulates in interfaces (preferential interaction) and perturbs water structure (chaotropic effect). Hence, it promotes protein unfolding [11], demicellization [20,21], and inhibits surfactant clouding [21] by facilitating the exposure of hydrophobic surfaces to water. It stabilizes the fluid lipid bilayer with its relatively large interface and hydration against transitions to gel and inverse hexagonal phases [14,17,22]. By relaxing the interfacial tension of membranes it also renders otherwise detergent-resistant gel phases of long-chain saturated lipids susceptible for the insertion of Triton, followed by solubilization [23] and it reduces the amount of octyl glucoside that is required for complete membrane solubilization [20]. Urea has been used as a standard tool in (un)folding studies of globular proteins and there is evidence that also membrane protein stability can be modulated by urea [24].

To shed more light at the effect of glycerol and urea on lipid–detergent systems, we have studied their impact on the self-association (e.g., micelle formation), membrane partitioning, and membrane solubilization by $C_{12}EO_8$ and, in some cases, octyl glucoside (OG). All these phenomena can very precisely and conveniently be measured by isothermal titration calorimetry applying the demicellization [25–28], uptake and release [29], and solubilization and reconstitution [30] protocols. Fluorescence and light scattering data reveal the accompanying structural changes.

2. Materials and methods

2.1. Materials

Non-ionic detergents octaethyleneglycol mono-dodecylether ($C_{12}EO_8$) and n-octyl-β-$_D$-glucopyranoside (OG) were obtained from Anatrace Inc., Maumee, OH in Anagrade purity (99% HPLC). The lipid, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) was purchased from Avanti Polar Lipids, Alabaster, AL. Glycerol, a Bioshop Canada product with purity>99%, and Urea (99.5% purity) from Sigma-Aldrich were used without further purification. Diphenyl hexatriene (DPH) was from Molecular Probes (Invitrogen), Eugene OR. Solvents were prepared by mixing desired volumes of glycerol or weights of urea with water from a Millipore system. Volume fractions, ϕ can be converted into molar concentrations, C, according to (subscript U for urea, but eq. holds for glycerol analogously):

$$\phi = \frac{V_U}{V_{total}} = C_U\,\bar{V}_U \tag{1}$$

where V_U denotes the volume of urea added to the mixture of total volume V_{total}, C_U represents the molar concentration of urea, and $\bar{V}_U = 43.2$ mL/mol the partial molar volume of urea in aqueous solution. For glycerol we find $\bar{V}$ to range from ≈70.8 to 73.1 mL/mol for $\phi_G = 0$–1, respectively [31,32]. In other words, 1v% cosolvent corresponds to 0.23 M of urea or 0.14 M of glycerol, respectively.

2.2. Liposome preparation

Vesicles were prepared as described [29,30,33], simply replacing the buffer hydrating the dry lipid by one with the desired content of co-solvent. Briefly, a solution of POPC in chloroform was dried under a stream of nitrogen gas and under vacuum and the dry lipid film weighed and dispersed in the desired solution of glycerol or urea (0–40v%). Each suspension was vortexed and freeze-thawed for five cycles before large unilamellar vesicles (LUVs) of the desired diameter were prepared by extrusion through Nuclepore filters of ~100 nm pore size in a Lipex extruder (Northern Lipids, Canada). Spot checks of the final lipid concentration using an inorganic phosphorus assay (Biovision, Inc.) confirmed an effective molecular weight of POPC of about 778 g/mol (the formula weight is 760 g/mol, i.e., about one water molecule remains bound after drying) and ruled out a significant loss of lipid upon extrusion in water and urea/water. We assume this holds true also in glycerol/water where the assay failed to yield stable results. The size of LUVs was confirmed to be close to the pore size by dynamic light scattering.

2.3. Isothermal titration calorimetry

The measurements were performed using a VP isothermal titration calorimeter [34] produced by MicroCal Inc., Northampton MA applying the protocols explained below. In all cases, concentrations of titrant and original cell content during the titration were corrected for the displacement of some sample from the totally filled cell as detailed by the manufacturer. Injection volumes ranged between 2 and 15 μL and were, typically, increased during a titration to ensure optimum resolution at low titrant concentration while keeping the total time of the run acceptable. All experiments were done at 25 °C.

2.3.1. Demicellization protocol [25–27]

The injection syringe of the calorimeter was loaded with micellar dispersions of $C_D^{syr} = 3$ mM ($C_{12}EO_8$) or 500 mM (OG), respectively, in water–cosolvent mixtures of the desired composition. The cell was filled with the corresponding water–cosolvent mixture (same batch). The syringe content was titrated into the cell in a series of injections and the heat of re-equilibration of the cell content after each injection, Q_{obs}, was recorded as a function of the average detergent concentration in the cell during the respective injection, C_D. Typically, $Q_{obs}(C_D)$ shows a sigmoidal change from the concentration range below to that above the CMC, which is identified as the point of inflection (maximum or minimum of first derivative). The heat of demicellization, Q_{demic}, is estimated as the difference between the Q_{obs} curves below- and above the CMC, respectively, linearly extrapolated to the CMC (see Fig. 1 below for a graphical representation). Then, the molar enthalpy change of transfer of detergent from the aqueous solution into micelles, $\Delta H_D^{aq\rightarrow m}$, is calculated as:

$$\Delta H_D^{aq\rightarrow m} = -Q_{demic}\frac{C_D^{syr}}{C_D^{syr} - CMC} \tag{2}$$