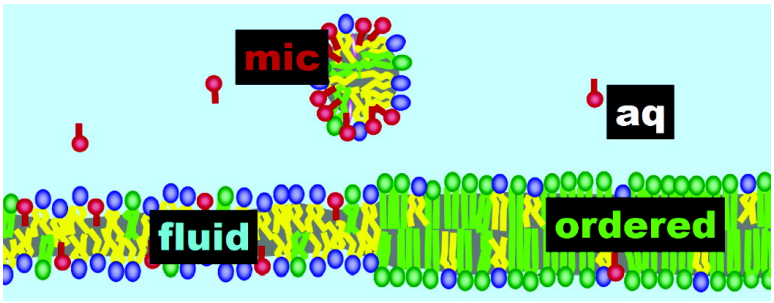
Article

A Quantitative Model Describing the Selective Solubilization of Membrane Domains

Sandro Keller, Alekos Tsamaloukas, and Heiko Heerklotz

J. Am. Chem. Soc., **2005**, 127 (32), 11469-11476• DOI: 10.1021/ja052764q • Publication Date (Web): 23 July 2005



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 2 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
ARTICLES
Published on Web 07/23/2005

A Quantitative Model Describing the Selective Solubilization of Membrane Domains

Sandro Keller,† Alekos Tsamaloukas,‡ and Heiko Heerklotz*,‡

Contribution from the Research Institute of Molecular Pharmacology FMP, Robert-Rössle-Strasse 10, 13125 Berlin, Germany, and the Biozentrum der Universität Basel, Klingelbergstrasse 50/70, 4056 Basel, Switzerland

Received April 28, 2005; E-mail: heiko.heerklotz@unibas.ch

Abstract: The classical three-stage model of membrane solubilization, including mixed membranes, membrane−micelle coexistence, and mixed micelles, is not applicable to demixed, domain-forming membranes and must, therefore, fail to describe the phenomenon of detergent-resistant membranes (DRMs). In lack of a quantitative model, it has often been assumed that ordered, detergent-depleted domains are inert, whereas fluid domains are solubilized. We establish a quantitative model based on equilibrium thermodynamics that is analogous to the three-stage model but comprises three components (two lipids and one detergent) in four phases (liquid-ordered and liquid-disordered membranes, micelles, and detergent in aqueous solution). For a given set of total concentrations and input parameters (initial abundance of ordered domains, solubilization boundaries of the pure lipids, etc.), it serves to calculate the phase boundaries and partial concentrations of all components in all phases. The results imply that the abundance and composition of ordered domains may vary substantially upon addition of detergent, both before and during solubilization of the fluid phase. It seems that gel-phase or order-preferring lipids are thermodynamically "resistant" regardless of the presence of a second, fluid phase. However, thermodynamic or kinetic resistance is not sufficient for obtaining DRMs because the resistant particles may be too small to be isolated. Cholesterol may be crucial for rendering the fragments large enough and, furthermore, enhance the formation of ordered domains by nonideal interactions with the detergent.

Introduction

The solubilization of biological membranes by detergents has long been used as the main method for the isolation and purification of membrane proteins and other constituents. Recently, considerable interest has been attracted by the finding that biological membranes can be solubilized selectively. Certain membrane constituents are incorporated into small micelles, whereas others remain in so-called detergent-resistant membrane fragments that are large enough to be separated by centrifugation.[1] This provides a unique tool to preselect classes of proteins to be isolated and to study the preferences of proteins for certain membrane environments, and it will undoubtedly be of great value also for future large-scale studies of membrane proteins. Apart from that, it has stimulated the hypothesis that the resistant fragments resemble functional domains, so-called "lipid rafts", existing already in the original, detergent-free membrane. Thousands of studies addressing this issue have been published.[2−5]

The solubilization of homogeneous fluid membranes is described by what is often referred to as the three-stage model.[6−8] In the first stage, detergent micelles added to a lipid membrane dispersion dissolve into monomers, and the latter partition between membrane and aqueous solution. When a critical detergent mole fraction, X_e^{sat}, is reached in the membrane, mixed micelles appear in coexistence with these saturated membranes during the second stage. The mole fraction of detergent in the micelles is X_e^{sol}. Addition of more detergent increases the number of micelles at the expense of membranes but leaves their internal compositions, X_e^{sol} and X_e^{sat}, unchanged. When the average detergent mole fraction in micelles and membranes, X_e, approaches X_e^{sol}, the last membranes disappear, and at $X_e > X_e^{sol}$, only mixed micelles are left in the third stage. This behavior can be well explained in terms of a simple thermodynamic model if mixed micelles, membranes, and the aqueous solution of detergent are considered thermodynamic pseudophases. This is a good approximation in most cases, although phases in the strict sense are homogeneous and separated macroscopically. Major deviations have been found for charged detergents forming very small micelles, such as bile salts,[9] where long-range micelle−micelle interactions and the entropy of mixing of micelles in the dispersion give rise to

† Research Institute of Molecular Pharmacology FMP.
‡ Biozentrum der Universität Basel.

(1) Brown, D. A.; Rose, J. K. *Cell* **1992**, *68*, 533−544.
(2) Simons, K.; Ikonen, E. *Nature* **1997**, *387*, 569−572.
(3) Edidin, M. *Annu. Rev. Biophys. Biomol. Struct.* **2003**, *16*, 257−283.
(4) Munro, S. *Cell* **2003**, *115*, 377−388.
(5) Simons, K.; Vaz, W. L. *Annu. Rev. Biophys. Biomol. Struct.* **2004**, *33*, 269−295.
(6) Helenius, A.; Simons, K. *Biochim. Biophys. Acta* **1975**, *415*, 29−79.
(7) Lichtenberg, D.; Opatowski, E.; Kozlov, M. M. *Biochim. Biophys. Acta* **2000**, *1508*, 1−19.
(8) Lichtenberg, D. *Biochim. Biophys. Acta* **1985**, *821*, 470−478.
(9) Hildebrand, A.; Neubert, R.; Garidel, P.; Blume, A. *Langmuir* **2002**, *18*, 2836−2847.

10.1021/ja052764q CCC: $30.25

significant contributions to the free energy of the system. A refined model has been established to account for such effects.[10]

Selective solubilization and detergent resistance cannot be treated in terms of the three-stage model. Rafts[2,5] are often assumed to be basically equivalent to liquid-ordered domains forming in certain lipid mixtures.[11−13] The equivalence of detergent-resistant membranes (DRMs) with rafts has been claimed on the basis that the detergent virtually does not insert into ordered membrane domains. Thus, one is tempted to conclude that the ordered domains are inert against the detergent and the fluid domains behave as predicted by the three-stage model. However, this reasoning neglects that changing one phase in an equilibrium affects the other phases, as well. For example, if a fluid-phase-preferring molecule (the detergent) is added, it should tend to shift the equilibrium in favor of the fluid phase (regardless of where it is localized). In a sophisticated qualitative consideration, London and Brown[14] mentioned other critical issues. For example, DRMs might overestimate the amount of ordered phase because they are usually isolated at low temperature or form by selective solubilization of certain lipids from a homogeneous intermediate state. Experimental data imply that the detergent may promote the formation of ordered domains already before solubilization and suggest nonideal interactions between detergents and order-preferring lipids as a possible driving force for such an effect.[15,16] Using neutron scattering, Nicolini et al.[17] have detected changes in domain size induced by detergents. Van Rheenen et al.[18] have provided evidence that detergent-induced domain formation as suggested by model studies occurs also in vivo, and that minute amounts of Triton lead to phosphatidylinositol 4,5-bisphosphate (PIP_2) clusters that do not exist in detergent-free cell membranes. On the basis of such findings, a more critical view of the "DRM = raft" hypothesis has been acquired recently,[3−5] but the problem still suffers from a lack of quantitative understanding.

Our aim is to make a first step toward such a quantitative understanding. We present a model describing the effects of a detergent on membrane domains before and during selective solubilization by extending the classical three-stage model, taking into account a third component and a fourth phase. On this level of complexity, a systematic approach requires starting with the simplest case, which is ideal mixing of the components in all phases. We will show that this basic case provides important insight and yields useful rules for such systems. However, we will also have to accept that this model is not sophisticated enough to account for all key properties of real multicomponent membranes. To illustrate the substantial influence of nonideal mixing, we introduce a single nonideality parameter into our model at the end of the study.

Theory

The fom Model for Ideal Mixing. The model applies to a thermodynamic equilibrium of two lipids, L1 and L2, and one detergent, D, during "**fom**" coexistence, that is, forming fluid (**f**) and ordered (**o**) membranes, micelles (**m**), and aqueous solution (**aq**, only for the detergent). The criterion for a system to be in equilibrium is that no transfer of any molecule from one phase to another can further reduce the Gibbs free energy of the system, meaning that the chemical potential of each component must be equal in all coexisting phases. For the detergent in the **fom** range, ideal mixing in all phases yields

$$\mu_D^{0,o} + RT \ln X_D^o = \mu_D^{0,f} + RT \ln X_D^f = \mu_D^{0,m} + RT \ln X_D^m = \mu_D^{0,aq} + RT \ln X_D{}^{aq} \quad (1)$$

The chemical potential comprises a constant standard value, μ_D^0, and a composition-dependent term, $RT \ln X_D^p$, containing the contribution from the entropy of ideal mixing. R denotes the universal gas constant, T the absolute temperature, and X_D^p the mole fraction of detergent in a phase **p** = **o**, **f**, **m**, or **aq**. We may rewrite eq 1 and the equivalent equations for the lipids, L1 and L2, using partition coefficients of a component C between the phases **p1** and **p2**, $K_C^{p1/p2}$, as

$$\mu_C^{0,p1} - \mu_C^{0,p2} = -RT \ln \frac{X_C^{p1}}{X_C^{p2}} \equiv -RT \ln K_C^{p1/p2} \quad (2)$$

Writing the mole fractions in terms of molar concentrations, c_C^p, yields six independent equations of the type

$$K_C^{p1/o} = \frac{c_C^{p1}(c_D^o + c_{L1}^o + c_{L2}^o)}{(c_D^{p1} + c_{L1}^{p1} + c_{L2}^{p1})c_C^o} \quad (3)$$

for the components C = L1, L2, or D and phases **p1** = **f** or **m** and **p2** = **o** (i.e., $K_D^{f/o}$, $K_D^{m/o}$, $K_{L1}^{f/o}$, $K_{L1}^{m/o}$, $K_{L2}^{f/o}$, and $K_{L2}^{m/o}$). For the aqueous detergent solution

$$K_D^{aq/o} = \frac{c_D^{aq}(c_D^o + c_{L1}^o + c_{L2}^o)}{55.5\ \mathrm{M} \times c_D^o} \quad (4)$$

Since these equations still describe mole fraction partition coefficients, the standard states remain hypothetical pure phases ($X_C^p = 1$) rather than 1 M solutions. The ordered phase is chosen as the reference state merely for technical reasons (**o** is present in many phase ranges) and without restriction of generality. All other $K_C^{p1/p2}$ values depend on the seven **o**-based partition coefficients given by eqs 3 and 4.

Trivially, the sum of all partial concentrations of a component (all referring to the total volume) must equal the total concentration, yielding another three independent equations, such as

$$c_D = c_D^o + c_D^f + c_D^m + c_D^{aq} \quad (5)$$

for c_D and analogous expressions for c_{L1} and c_{L2}. The system of eqs 3−5 represents the model used here; all 10 equations are given explicitly in the Supporting Information. Selecting appropriate values for the seven $K_C^{p1/o}$ values and the three total concentrations, c_C, yields 10 equations with 10 unknown variables (c_C^p with C = D, L1, or L2 and **p** = **f**, **o**, **m**, and, for D, also **aq**), so that an unequivocal solution can be determined

(10) Roth, Y.; Opatowski, E.; Lichtenberg, D.; Kozlov, M. M. *Langmuir* **2000**, *16*, 2052−2061.
(11) McMullen, T. P. W.; Lewis, R. N. A. H.; McElhaney, R. N. *Curr. Opin. Colloid Interface Sci.* **2004**, *8*, 459−468.
(12) Veatch, S. L.; Polozov, I. V.; Gawrisch, K.; Keller, S. L. *Biophys. J.* **2004**, *86*, 2910−2922.
(13) Ipsen, J. H.; Mouritsen, O. G.; Zuckermann, M. J. *Biophys. J.* **1989**, *56*, 661−667.
(14) London, E.; Brown, D. A. *Biochim. Biophys. Acta* **2000**, *1508*, 182−195.
(15) Heerklotz, H. *Biophys. J.* **2002**, *83*, 2693−2701.
(16) Heerklotz, H.; Szadkowska, H.; Anderson, T.; Seelig, J. *J. Mol. Biol.* **2003**, *329*, 793−799.
(17) Nicolini, C.; Thiyagarajan, P.; Winter, R. *Phys. Chem. Chem. Phys.* **2004**, *6*, 5531−5534.
(18) van Rheenen, J.; Achame, E. M.; Janssen, H.; Calafat, J.; Jalink, K. *EMBO J.* **2005**, *24*, 1664−1673.

numerically using the Solver (Frontline Systems, Incline Village, USA) function in an Excel spreadsheet (Microsoft, Redmond, USA).

The phase model used here implicitly refers to large domains, that is, macroscopically separated phases with a negligible mixing entropy of the different domains and negligible interfacial energies of the borders between the domains. In case of weak line tension between the different domains, the latter become small, thus gaining some additional entropy and making lipid sorting somewhat more favorable.

The Models for the Phase Ranges fo, om, f, m, and fm. If, for the selected set of parameters, the system does not show **fom** coexistence, at least one of the resulting partial concentrations becomes negative, indicating that the **fom** model is not appropriate in the respective concentration range. A model for **fo** coexistence (membranes with fluid and ordered domains and detergent monomers, but no micelles) is obtained by omitting the equations for $K_D^{m/o}$, $K_{L1}^{m/o}$, $K_{L2}^{m/o}$, and setting $c_C^m = 0$ for all components, C. The model for **om** coexistence is derived analogously by omitting all equations and concentrations for the **f** phase. For the **f** phase range (only fluid membranes and aqueous detergent monomers), the equilibrium is given by $K_D^{aq/o}/K_D^{f/o}$ (cf. eq 3 with **p1** = **f**, C = D, and eq 4), and analogous expressions hold for the **m** range (only micelles and aqueous detergent monomers).

For **fm** (solubilization of fluid membranes in the absence of ordered domains), we have to change the reference state of our K values to **f**, deriving $K_C^{m/f} = K_C^{m/o}/K_C^{f/o}$ and an analogous equation for $K_D^{aq/f}$. The model then simplifies to the classical three-stage case (**f**, **fm**, and **m**) for $c_{L2} = 0$ and $c_C^o = 0$ for all components, C.

Input Parameters. Unfortunately, some of the seven partition coefficients used here are not straightforward to be estimated or measured. We therefore chose another, more illustrative set of input parameters that serves to calculate these seven K values (cf. the Supporting Information for conversion rules).

(1) The critical micellar concentration (CMC) of the detergent determines its partitioning between the micellar and the aqueous phase, $K_D^{m/aq}$, and, with $K_D^{m/o}$ (derived below), also $K_D^{aq/o}$.

(2,3) The effective mole fractions at the onset and completion of solubilization of the fluid-phase-preferring lipid L1, X_e^{sat}(L1) and X_e^{sol}(L1), yield $K_D^{m/f}$ and $K_{L1}^{m/f}$ and, with $K_D^{m/o}$ and $K_{L1}^{f/o}$ (derived below), also the basic $K_D^{f/o}$ and $K_{L1}^{m/o}$. As standard values, we chose $X_e^{sat}(L1) = 0.29$ and $X_e^{sol}(L1) = 0.63$ as found for Triton X-100/1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) at 37 °C.[16] The somewhat higher values reported for the partitioning of Triton[19−21] and most other strong detergents into POPC or egg lecithin at room temperature[22,23] would lead to the same general behavior.

(4,5) Analogously, we select the onset and completion of solubilization of a pure ordered membrane consisting of L2, X_e^{sat}(L2) and X_e^{sol}(L2), which yield $K_D^{m/o}$ and $K_{L2}^{m/o}$. These parameters are not straightforward to be measured because solubilization of ordered phases may implicitly include the formation of disordered phases. We varied these parameters over a broad range to obtain general conclusions without experimental data (cf. Results).

(6) We define the fraction of ordered lipid as

$$\xi^o \equiv \frac{c_{L1}^o + c_{L2}^o}{c_{L1} + c_{L2}} \tag{6}$$

The value of ξ^o depends on lipid composition, temperature, pressure, and detergent content. We select the fraction of ordered lipid in the absence of detergent, $\xi^o(0) \equiv \xi^o(X_e = 0)$ as another input parameter. With increasing temperature, the ordered phase melts, and ξ^o varies from unity to zero. The progress of the thermotropic transition can, for instance, be measured by scanning calorimetry.

(7) One basic partition coefficient, the affinity of L2 to the fluid as compared with the ordered phase, $K_{L2}^{f/o}$, is specified as an input parameter. Without restriction of generality, we assume that L2 prefers the ordered phase, $K_{L2}^{f/o} < 1$. Both $K_{L2}^{f/o}$ and $\xi^o(0)$ serve to calculate $K_{L1}^{f/o}$, and the requirement that $K_{L1}^{f/o} > 0$ limits the range for choosing $K_{L2}^{f/o}$ at a given $\xi^o(0)$.

Using these seven input parameters and the total concentrations, c_{L1} and c_{L2}, we calculate the solutions for increasing c_D, corresponding to a titration of detergent into the lipid dispersion. If the results are, however, plotted as a function of the effective detergent mole fraction in aggregates, defined as

$$X_e \equiv \frac{c_D - c_D^{aq}}{c_D - c_D^{aq} + c_{L1} + c_{L2}} \tag{7}$$

they will agree for all calculations sharing a certain L2-to-L1 molar ratio, c_{L2}/c_{L1}, regardless of the absolute concentrations, c_{L1} and c_{L2}. We chose an equimolar mixture for all cases presented here.

Results

Typical Behavior of Predominantly Ordered Lipid Mixtures. Figure 1 shows the results of a typical model calculation for the input parameters given in the legend. The results for the partial concentrations of the components, C, in the phases, **p**, are plotted as percentages of the total concentration, $\xi_C^p \equiv c_C^p/c_C$. The **fom** model returns positive solutions for the range of 0.31 mM < c_D < 0.73 mM (0.15 < X_e < 0.37). At lower detergent content, the system is in **fo** equilibrium. Over a large concentration range of 0.73 mM < c_D < 4.5 mM (0.37 < X_e < 0.81), "resistant" ordered membrane particles coexist with micelles (**om**), and only at very high detergent concentrations of c_D > 4.5 mM is the membrane fully solubilized (**m**). The consistency of the solution is illustrated by the fact that the partial concentrations vary continuously at the phase boundaries. For example, for c_D = 0.31 mM (**fo**|**fom** boundary), the model for **fo** as well as that for **fom** yield the same result. The data given in Figure 1 are plotted as a function of the absolute detergent concentration, c_D, in Figure S1 in the Supporting Information.

The results show that in the **fo** range, a considerable amount of L1 but also a little L2 is transferred from **o** into **f**, that is, ordered domains or part of them are converted into fluid ones, and the remaining **o** domains are enriched in L2. The ordered domains are further changed upon selective solubilization of

(19) Paternostre, M. T.; Roux, M.; Rigaud, J. L. *Biochemistry* **1988**, *27*, 2668−2677.
(20) Partearroyo, M. A.; Alonso, A.; Goñi, F. M.; Tribout, M.; Paredes, S. *J. Colloid Interface Sci.* **1996**, *178*, 156−159.
(21) Kragh-Hansen, U.; le Maire, M.; Møller, J. V. *Biophys. J.* **1998**, *75*, 2932−2946.
(22) Heerklotz, H.; Seelig, J. *Biophys. J.* **2000**, *78*, 2435−2440.
(23) Heerklotz, H. In *Phospholipids Handbook*, 2nd ed.; Cevc, G., Ed.; Plenum Press: New York, 2005.

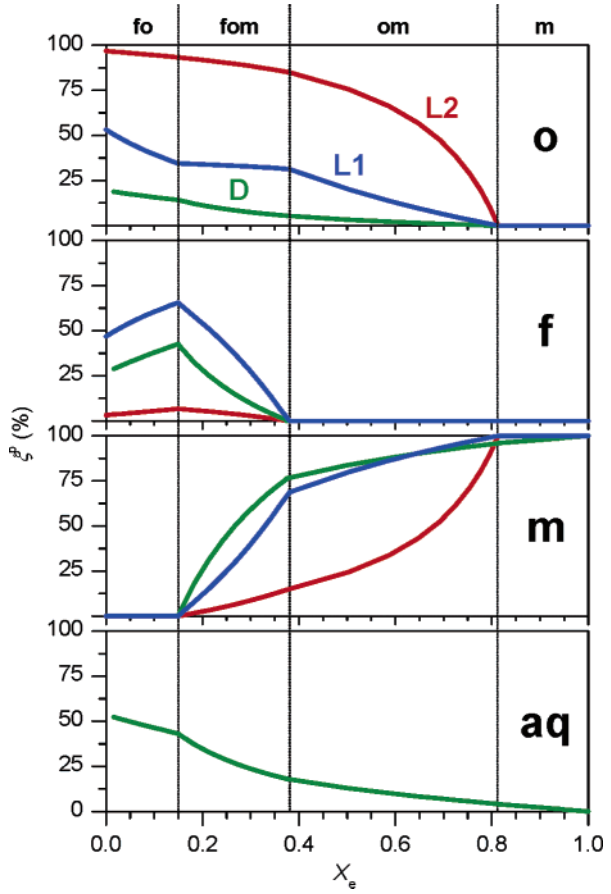


Figure 1. Results of a simulation for fixed lipid concentration ($c_{L1}/c_{L2} = 1$) and increasing detergent concentration given as effective mole fraction, X_e (abscissa). The input parameters are CMC = 0.23 mM, $X_e^{sat}(L1) = 0.29$, $X_e^{sol}(L1) = 0.63$, $X_e^{sat}(L2) = 0.10$, $X_e^{sol}(L2) = 0.90$, $\xi^o(0) = 75\%$, and $K_{L2}^{f/o} = 0.1$. Plotted are the fractions, ξ^p, of the two lipids, L1 and L2, and of the detergent, D, localized in each of the four possible phases (**p**): ordered (**o**) and fluid (**f**) membranes, micelles (**m**), and aqueous solution (**aq**). Phase boundaries are recognized by the appearance or disappearance of phases and indicated by dotted lines.

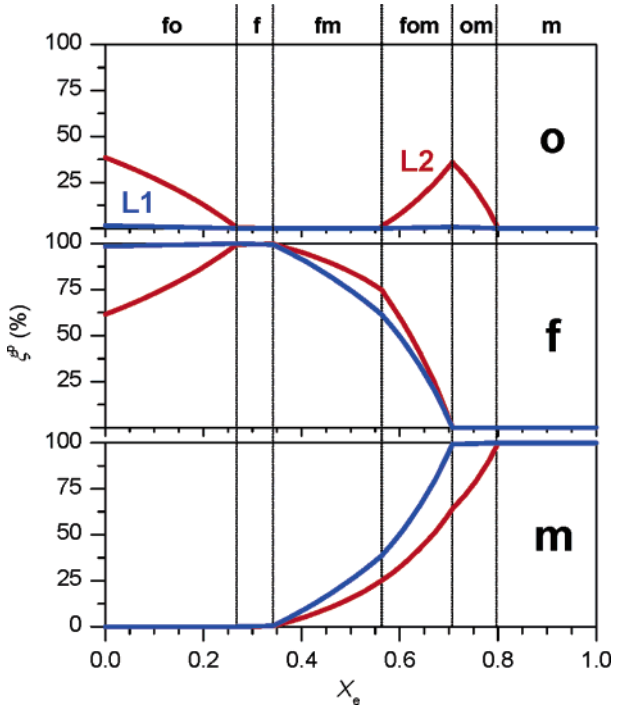


Figure 2. The fractions, ξ^p, of the lipids, L1 and L2, in the ordered (**o**), fluid (**f**), and micellar (**m**) phase, and the phase ranges as a function of the effective mole fraction of detergent in the system, X_e. Ordered domains are disintegrated by addition of detergent to the membrane until **fo**|**f** but reappear upon selective solubilization of L1 at the **fm**|**fom** boundary. The input parameters are the same as in Figure 1 with the exception of $\xi^o(0) = 20\%$ and $K_{L2}^{f/o} = 0.4$. The results for the detergent are not shown.

the **f** phase (**fom**). In the **fom** and **om** ranges, the amount of L2 in **o** domains decreases nearly linearly with the total detergent concentration (cf. Figure S1 in the Supporting Information), and the disappearance of fluid membranes at the **fom**|**om** boundary has only a minor effect on the solubilization of L2. The aqueous detergent concentration, c_D^{aq}, increases continuously with increasing total c_D and approaches the CMC for large c_D. However, the fraction of detergent that is in aqueous solution decreases, so that the effective mole fraction, X_e, approaches the total mole fraction. When it comes to discussing the extraction of DRMs, which is usually done at high concentrations ($c_D \gg$ CMC), we can ignore the aqueous detergent and interpret X_e approximately as the total mole fraction of detergent in the system.

Typical Behavior of Predominantly Fluid Membranes. Figure 2 illustrates a calculation for a system that can be imagined to represent a higher temperature, where most of the ordered domains are "molten". We assume a relatively small fraction of ordered domains in the detergent-free membrane, $\xi^o(0) = 20\%$, and a strong sorting of L1 into disordered domains, $K_{L2}^{f/o} = 0.4$. Addition of detergent converts ordered domains progressively into fluid ones until an **fo**|**f** boundary is reached, beyond which only homogeneous fluid membranes are left. After the appearance of micelles at X_e(**f**|**fm**), there is a preferential solubilization of L1 because the order-preferring lipid, L2, has a weaker affinity to micelles. As a consequence, the remaining membranes are enriched in L2, and at a critical L2-to-L1 ratio, ordered domains reappear (**fm**|**fom** boundary). In the **fom** range, the lipids from fluid domains are sorted into micelles (L1) and ordered domains (L2). At **fom**|**om**, also the ordered domains start to be solubilized, and this process is completed at virtually the same X_e(**om**|**m**) as in Figure 1 describing the solubilization of an originally much more ordered membrane.

General Phase Behavior. Let us, at first, inspect the effect of the preferences of the lipids, L1 and L2, for fluid versus ordered phases on the solubilization behavior. The input parameters describing these properties are $K_{L2}^{f/o}$ and $\xi^o(0)$. With $\xi^o(0)$ decreasing from unity (all ordered) to zero (all fluid), Figure 3 bears some resemblance to a phase diagram since the **o** phase melts with increasing temperature. The boundaries are distorted because $\xi^o(0)$ is not a linear function of T, and the other input parameters may also depend somewhat on temperature,[23] which is not considered here.

The bottom panel of Figure 3 was obtained for a strong preference of L2 for the **o** phase as quantified by $K_{L2}^{f/o} = 0.1$. From $\xi^o(0) = 100\%$ (corresponding to low temperature) to $\xi^o(0) \approx 40\%$ (thermotropic transition range), we find the same sequence of phase ranges as illustrated in Figure 1 for $\xi^o(0) = 75\%$, that is, **fo**−**fom**−**om**−**m**. Progressive melting of the ordered domains shifts the onset of micelle formation (**fo**|**fom**) and, particularly, the complete solubilization of the fluid phase (**fom**|**om**) to higher detergent contents. This is logical because there is more lipid in the **f** phase to be solubilized in the first

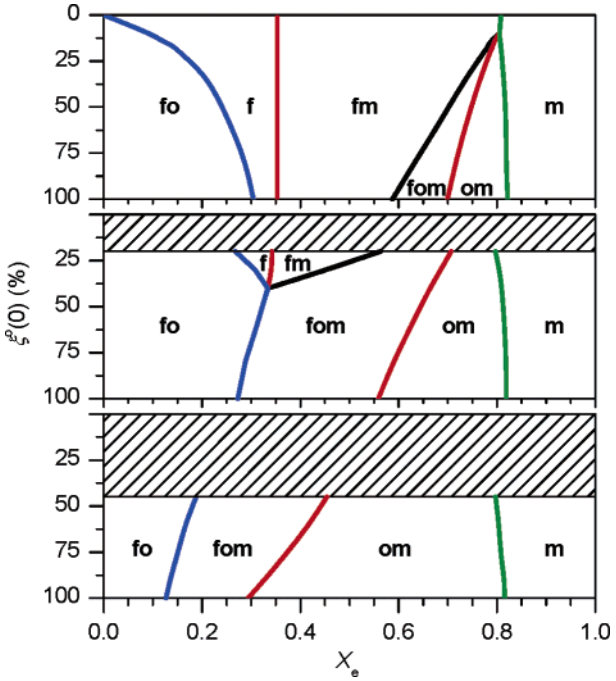


Figure 3. Phase boundaries, X_e, for various degrees of order in the original membrane, $\xi^o(0)$ (ordinates), and partition coefficients of L2: top panel, $K_{L2}^{f/o} = 0.5$; center, 0.4; bottom, 0.1. The other input parameters are the same as in Figure 1. Hatched areas are not accessible because they would correspond to negative $K_{L1}^{f/o}$ values.

place. However, the detergent concentration needed to finally solubilize the "resistant" **o** phase is almost independent of whether the lipid was originally in an **o** or in an **f** phase.

Having more than ~60% of the lipid in the **f** phase is incompatible with a strong preference of L2 for the **o** phase. Systems with less **o** must have higher $K_{L2}^{f/o}$ values; the center panel of Figure 3 uses a value of $K_{L2}^{f/o} = 0.4$. At high initial order ($\xi^o(0) > 50\%$), we obtain the same phase ranges as for $K_{L2}^{f/o} = 0.1$ (bottom panel), but with the phase boundaries shifted to higher detergent contents. At $\xi^o(0) \approx 40\%$, the detergent abolishes all ordered domains already before solubilization starts. Systems with only a few ordered domains already in the absence of detergent ($\xi^o(0) < 20\%$) require even larger values of $K_{L2}^{f/o}$ (top panel). The higher affinity of L2 to the fluid phase shifts the pattern in favor of the fluid phase. Now, intermediate **f** and **fm** phase ranges are obtained already from originally largely ordered membranes (high ξ^o). For $\xi^o(0) < 12\%$, ordered domains cease to reappear. As disorder-preferring detergents cannot promote ordered domains in the case of ideal mixing, the phase behavior must approach that of homogeneous fluid membranes (three-stage model with **f**, **fm**, and **m**) for vanishing ordered domains. The thermodynamic resistance of L2 against solubilization, which is quantified by X_e(**om**|**m**), is virtually independent of $\xi^o(0)$ as well as of $K_{L2}^{f/o}$.

Effects of the Detergent and Fluid Lipid. The interactions of the detergent with the more fluid lipid, L1, are characterized by the CMC, X_e^{sat}(L1), and X_e^{sol}(L1). We compared calculations based on parameters typical of POPC/Triton X-100 (TX; CMC = 0.23 mM, X_e^{sat}(L1) = 0.29, X_e^{sol}(L1) = 0.63) with those of octyl glucoside (OG; 22 mM, 0.61, 0.76).[24,25] OG is a weak detergent that usually fails to yield DRMs. The fact that the CMC of OG is much larger has the consequence that a higher absolute detergent concentration is needed for complete solubilization of the ordered phase, that is, 4.5 mM for TX but 22 mM for OG. However, relative to the CMC, there is more TX needed (~20×CMC) than OG (1×CMC), so that an isolation procedure using, for example, twice the CMC could yield DRMs with TX but not with OG.

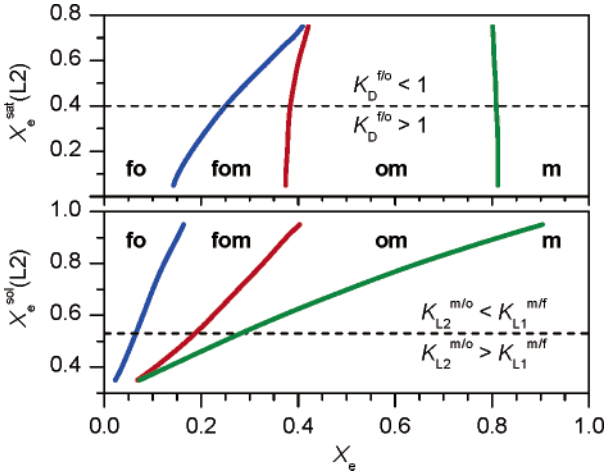


Figure 4. The effect of the onset and completion of solubilization of pure ordered L2, X_e^{sat}(L2) and X_e^{sol}(L2), on the phase ranges. For the case of detergents added to detergent-resistant membranes, **f** is more susceptible to solubilization (corresponding to X_e^{sol}(L2) > 0.53), and the detergent inserts preferably into **f** (corresponding to X_e^{sat}(L2) < 0.40). All other input parameters are selected as in Figure 1.

The much higher X_e^{sat} and X_e^{sol} values of OG compared with those of TX also shift the solubilization of the fluid phase in the L1/L2 mixture to higher X_e values: X_e(**fo**|**fom**) from 0.15 (TX) to 0.40 (OG) and X_e(**fom**|**om**) from 0.37 (TX) to 0.57 (OG). The fraction of lipid in the ordered phase, starting at 75% in the calculation, decreases to 59% upon solubilization of the fluid phase at X_e(**fom**|**om**) with TX but to 39% with OG. The enrichment of the **o** phase with L2 ($c_{L2}^o/c_{L1}^o = 1.8$ before addition of detergent) at this point is stronger for OG (5.5) than for TX (2.7). It appears that the effects within **fo** are generally enhanced for OG, which has a much broader **fo** range. Finally, we note that nonideal interactions (cf. below) of weak detergents, such as OG,[22] with membranes are expected to be weaker, as well. The "resistance limit", X_e(**om**|**m**), is independent of the characteristic parameters of the fluid lipid varied here.

Effects of the Solubilization Behavior of L2. Figure 4 illustrates the influence of the solubilization behavior of lipid L2, which is quantified in terms of X_e^{sat}(L2) and X_e^{sol}(L2). These two input parameters are used to determine several characteristic partition coefficients, in particular $K_D^{f/o}$, which describes the affinity of the detergent to ordered versus fluid membrane domains, and $K_{L2}^{m/o}$, which indicates the tendency of L2 to become solubilized from ordered domains into micelles.

The top panel of Figure 4 keeps X_e^{sol}(L2) = 0.90 constant and increases X_e^{sat}(L2). This corresponds to a decrease of $K_D^{f/o}$ from 8 (at X_e^{sat}(L2) = 0.05) to 1 (0.40) and 0.6 (0.75). This means that X_e^{sat}(L2) > 0.40 corresponds to a detergent that prefers insertion into and formation of the ordered compared with the fluid phase, a condition that seems not to be relevant to the isolation of DRMs (but maybe to membrane additives

(24) Paternostre, M.; Meyer, O.; Grabielle-Madelmont, C.; Lesieur, S.; Ghanam, M.; Ollivon, M. *Biophys. J.* **1995**, *69*, 2476−2488.

(25) Keller, M.; Kerth, A.; Blume, A. *Biochim. Biophys. Acta* **1997**, *1326*, 178−192.

other than detergents). If pure L2 requires more detergent for the onset of solubilization, the L1/L2 mixture will do so, as well. The completion of solubilization of the **f** as well as of the **o** phase is, however, essentially unaffected.

Finally, we kept X_e^{sat}(L2) fixed at 0.10 and increased the detergent content required for the completion of solubilization of pure L2, X_e^{sol}(L2), from 0.35 to 0.98. Below $X_e^{sol}(L2) = 0.53$, $K_{L2}^{m/o}$ is larger than $K_{L1}^{m/f}$, meaning that the ordered domains will be preferentially solubilized, again a case that is not of interest here. The larger X_e^{sol}(L2), the more selective is the solubilization of the fluid phase, and the more will the detergent accumulate in the **f** and be repelled from the **o** phase. The consequences are shown in the bottom panel of Figure 4. Growing resistance of pure L2 against solubilization increases the amount of detergent that is needed for the onset of micelle formation in the mixture, X_e(**fo**|**fom**), for the complete solubilization of the fluid phase, X_e(**fom**|**om**), and, in particular, for the complete solubilization of the ordered phase, X_e(**om**|**m**). In fact, this is the only parameter that governs the thermodynamic resistance of the mixture, whereas all other parameters varied so far have virtually no influence on X_e(**om**|**m**).

Nonideal Mixing. In general, mixing in the membrane and micelle phases might possibly be nonideal, necessitating additional terms in the expressions for the chemical potentials. Here, we demonstrate the substantial effect of a single nonideality parameter for pairwise interactions between detergent and lipid L2 in the fluid phase. That this interaction is highly nonideal in DRM-forming systems is supported by the finding that the partition coefficient of Triton into largely fluid POPC/egg sphingomyelin (eSM)/cholesterol (1:1:1 mol/mol/mol) vesicles at 37 °C is about 6 times smaller than that into pure POPC.[16] Even though we cannot distinguish whether this nonideality is a consequence of the sphingomyelin or the cholesterol or both of them in a cooperative manner, we chose a moderately unfavorable nonideality parameter, $\rho_{D/L2}^{f} = 5$ kJ/mol, to assess the possible consequences of nonideal mixing. The parameter accounts for the excess free energy of the fluid phase as compared with ideal mixing, G_E^f, according to

$$G_E^f = \rho_{D/L2}^f X_D^f X_{L2}^f \tag{8}$$

The nonideal free energy, G_E^f, is much smaller than $\rho_{D/L2}^f$ because $X_D^f, X_{L2}^f \ll 1$. All other possible nonideality parameters were neglected.

Figure 5 presents the extreme case of a membrane at the completion of the melting of the ordered domains, so that $\xi^o(0) \approx 0$. Addition of detergent induces the formation of ordered domains, which are growing further beyond the **fo**|**fom** boundary by selective solubilization of L1. The sample would show >60% of the lipid as thermodynamically resistant, although it was all fluid before the addition of detergent.

Discussion

The Nature of Detergent Resistance. The isolation of a DRM fraction from a membrane sample depends on a number of properties that may be of kinetic, thermodynamic, or technical kind. Our study allows us to discuss DRMs if these are thermodynamically stable. Then, detergent resistance of a lipid means that it remains in a bilayer structure up to very high detergent contents or, in terms of our model, that it has a very high X_e(**om**|**m**). It turns out in all calculations that the amount of detergent required for solubilizing the "resistant" **o** domains depends

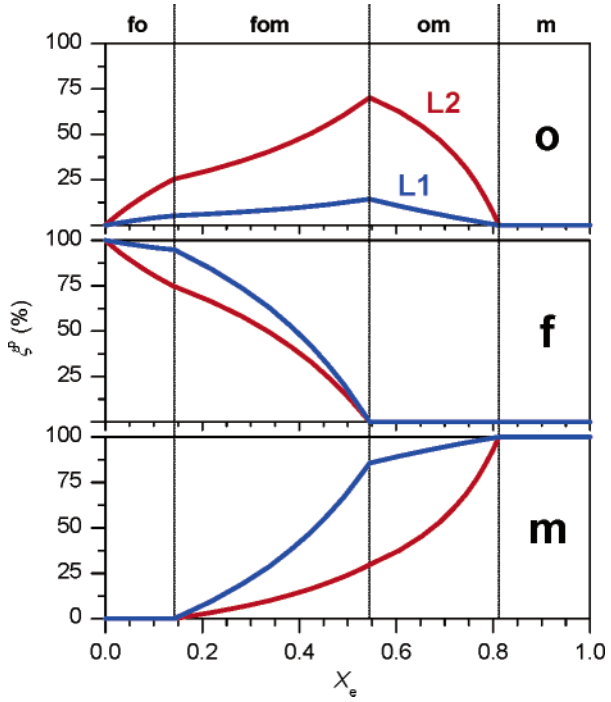


Figure 5. Effect of nonideal mixing between detergent and order-preferring lipid as quantified by a nonideality parameter, $\rho_{D/L2}^f = 5$ kJ/mol. The fraction of initially ordered membrane is $\xi^o(0) = 0.001$; the other input parameters are as in Figure 1. In the case of unfavorable nonideal mixing between D and L2 and at low initial degree of order, addition of D promotes the ordered phase.

almost exclusively on one input parameter, X_e^{sol}(L2). For a detailed interpretation of this behavior, it is advantageous to quantify the composition of the system in terms of effective mole ratios, R_e, rather than mole fractions, X_e, using the simple conversion $R_e = X_e/(1 - X_e)$. The D-to-L2 mole ratio in micelles at the disappearance of ordered membrane particles, $R_{D/L2}^m$(**om**|**m**), is virtually independent of the presence or absence of L1. In pure L2, this ratio is also termed R_e^{sol}(L2). We may thus write

$$R_{D/L2}^m(\mathbf{om}|\mathbf{m}) = R_e^{sol}(L2) = \frac{K_D^{m/o}(K_{L2}^{o/m} - 1)}{K_D^{m/o} - 1} \xrightarrow{K_D^{m/o}, K_{L2}^{o/m} \gg 1} K_{L2}^{o/m} \tag{9}$$

The expression of R_e^{sol}(L2) in terms of partition coefficients of L2 and D between **o** and **m** is derived in the Supporting Information. The approximation on the right-hand side of eq 9 is valid for large $K_D^{m/o}$ (detergent prefers **m** strongly over **o**) and large $K_{L2}^{o/m}$ (L2 prefers **o** strongly over **m**), which is typically fulfilled by order-preferring lipids. Thus, the resistance of L2 in any mixture with a more readily solubilizable lipid is solely determined by and equal to the preference of the resistant lipid, L2, for **o** over **m**. No property of L1 nor even its mere existence has any influence on the **om**|**m** phase boundary of the mixture.

A large $K_{L2}^{o/m}$ is expected if the transfer of the lipid into the micelle requires not only some change in interfacial curvature but also additional energetically costly transformations, such as chain melting or disordering of almost stretched chains well below the melting temperature. In light of this, all lipids forming gel or ordered phases at a given temperature must be expected to be resistant, both in mixtures with others and alone.

The latter suggestion seems to be in conflict with the finding that pure 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) or pure sphingomyelin does not necessarily yield DRMs.[26,27] However, thermodynamic (or maybe kinetic) resistance is only a necessary but not a sufficient criterion for the observation of DRMs. Additionally, the resistant particles must be large enough to be separated by centrifugation. The presence of cholesterol or other lipids may be important for DRMs not primarily by making the domains more resistant but by rendering the resistant particles larger. Furthermore, DRMs might also be thermodynamically unstable nonequilibrium structures that can be isolated because of the slow kinetics of equilibration.[14]

Solubilization of Membranes in the Gel Phase. As mentioned in the previous section, pure gel-phase lipids seem to be thermodynamically resistant, but the resistant membrane fragments may be too small to be detected or isolated. Funari et al.[28] have described, in fact, "gel-phase micelles", that is, small bilayer fragments of virtually pure DPPC gel phase surrounded by a hoop of detergent, which represent the thermodynamically resistant but technically soluble systems discussed here. We observed a similar behavior for mixtures of eSM and TX (Heerklotz et al., unpublished); the sample was optically clear below the melting point, T_m, of eSM but "melted" at $T_m = 39$ °C, with approximately the heat expected for pure eSM. The dispersion became turbid above T_m. This supports the hypothesis that most of the lipid is still in an almost detergent-free gel-phase bilayer below T_m (what we call "thermodynamically resistant"), but the bilayer fragments are too small to be detected by turbidity or centrifugation (i.e., "technically soluble"). Upon chain melting, the membrane loses its thermodynamic resistance, and the local detergent concentration at the edges decreases because the detergent distributes over the whole membrane. In turn, the fragments merge to form large membrane particles or vesicles.

These arguments and the analogy of the process to what our model reveals about mixtures also resolves the paradox of enhanced susceptibility to solubilization of membranes slightly below the melting temperature.[26,27,29] The chains in a gel phase are arranged to form a hexagonal lattice, and clusters of crystal-like packing are separated by line defects. A detergent or any other molecule that does not fit into the lattice is accumulated in the defect domains. This has, for example, long been known for pyrene, which shows an enhanced excimer formation in membranes somewhat below the melting temperature as it segregates into small areas at high local concentration.[30] If a detergent is added to a membrane, it will also accumulate in such less tightly packed domains. This preference will give rise to a very high local detergent content in the defect ranges, a growth of the defects, and most likely a splitting or shrinking of the ideally packed gel clusters, rendering them very small. Little detergent is required to let pieces of well-packed gel phase that are too small to make the sample turbid "fall apart" by covering their edges (cf. also London and Brown[14]). The same phenomenon may account for the effect of gangliosides,[29] if these, as often assumed, promote ordered phases, and of membrane-perturbing solutes, which may render gel-phase membranes technically soluble even far below the melting point.[26]

Line Tension and Domain Size: Cholesterol Might Enlarge Resistant Particles. As noted above, the phase equilibrium model used here does not consider the size of the domains, which is determined by the tradeoff between the entropy of mixing (favoring small domains) and the line tension of the domain borders (favoring shorter borders, i.e., larger domains). Hence, molecules reducing the line tension between the domains could abolish detectable DRMs by rendering them too small without affecting the actual equilibrium of the domains as described here. Nicolini et al.[17] have demonstrated by small-angle neutron scattering that Triton may reduce the domain size in model systems. On the contrary, an agent that enhances the line tension may serve to make DRMs large enough to be detectable. Cholesterol seems to be such a line tension modulator. Galla and Sackmann[30] have shown that addition of cholesterol to DPPC abolishes the coexistence of gel clusters and defect ranges, so that the segregation of the probe below T_m is eliminated. Hence, more detergent is expected to be required for the disintegration of the membrane, and the particles might be larger. The phase diagram of giant liposomes composed of 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC)/sphingomyelin/cholesterol established by Kahya et al.[31] provides crucial insight; domains in the gel−fluid coexistence range are too small to be visualized by confocal fluorescence microscopy and can only be detected by correlation spectroscopy. However, addition of ~20 mol % of cholesterol induces growth of the domains, making them visible in the microscope. In summary, it appears that lipids below their melting temperature may generally be detergent-resistant in the thermodynamic sense, regardless of whether they are mixed with cholesterol or other lipids. Cholesterol may, however, be necessary for rendering the resistant particles large enough to make them visible or separable by centrifugation.

Another possible role of cholesterol is to induce a marked nonideal mixing in the fluid phase. This would not affect the resistance of L2 given by $X_e(\mathbf{om}|\mathbf{m})$, but it could greatly increase the amount of resistant lipid, $\xi^o(\mathbf{om}|\mathbf{m})$, by detergent-induced formation of ordered domains, as illustrated in Figure 5. Very large concentrations of cholesterol can also eliminate visible domains in giant liposomes,[31,32] but this is often explained by the formation of a continuous liquid-ordered phase.[11,33]

Are Rafts Equivalent to DRMs? It has often been assumed that functional in vivo domains in detergent-free membranes (lipid rafts) and DRMs are both governed by an equilibrium of fluid and ordered lipid domains. Let us, for the sake of the argument, assume that this is so. Then, our model should yield the key properties of rafts at $X_e = 0$ and those of the corresponding DRMs at $X_e(\mathbf{fom}|\mathbf{om})$, where the fluid membrane domains are fully solubilized. The assumption of ideal mixing in all phases implies that only part of the rafts are actually obtained as DRMs at the same temperature ($\xi^o(\mathbf{fom}|\mathbf{om}) < \xi^o(0)$) and that the DRMs are enriched in L2 (c_{L2}/c_{L1} at **fom**|**om**

(26) Patra, S. K.; Alonso, A.; Goñi, F. M. *Biochim. Biophys. Acta* **1998**, *1373*, 112−118.
(27) Alonso, A.; Villena, A.; Goñi, F. M. *FEBS Lett.* **1981**, *123*, 200−204.
(28) Funari, S. S.; Nuscher, B.; Rapp, G.; Beyer, K. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 8938−8943.
(29) Sot, J.; Collado, M. I.; Arrondo, J. L. R.; Alonso, A.; Goñi, F. M. *Langmuir* **2002**, *18*, 2828−2835.
(30) Galla, H. J.; Sackmann, E. *Biochim. Biophys. Acta* **1974**, *339*, 103−115.
(31) Kahya, N.; Scherfeld, D.; Bacia, K.; Poolman, B.; Schwille, P. *J. Biol. Chem.* **2003**, *278*, 28109−28115.
(32) Feigenson, G. W.; Buboltz, J. T. *Biophys. J.* **2001**, *80*, 2775−2788.
(33) de Almeida, R. F.; Fedorov, A.; Prieto, M. *Biophys. J.* **2003**, *85*, 2406−2416.

is larger than at $X_e = 0$). Taking into account one nonideality parameter shows, however, that DRMs might also overestimate the amount of lipid in rafts and may, in the extreme case, be induced by the detergent in originally homogeneous fluid membranes.

Thus, the present model suggests that DRMs must be expected to differ markedly from rafts. There may, of course, be special cases where all the detergent effects cancel out each other, so that the ordered domains are, indeed, isolated without major changes. A set of parameters that minimizes the effects of the detergent on the abundance and composition of ordered domains up to the **fom**|**om** boundary (DRMs) is $\xi^o(0) = 40\%$, $K_{L2}^{f/o} = 0.2$, $X_e^{sol}(L2) = 0.93$, $\rho_{L2/D}^{f} = 2.5$ kJ/mol (other parameters as in Figure 1). The first two parameters describing the detergent-free system correspond to the case of an extremely strong sorting of the lipids between the domains, $K_{L1}^{f/o} = 21$. Consequently, only 3% of all L1 but 77% of all L2 resides in **o** domains (averaging to $\xi^o(0) = 40\%$) in the detergent-free system. This pronounced enrichment of **o** with L2 leaves practically no freedom for the general trend of the detergent to further enrich **o** in L2. The tendency to fluidize the membrane prior to micelle formation by adding fluid phase-preferring detergent (here, $K_D^{f/o} = 4.3$) is balanced by the weak nonideality parameter. $X_e^{sol}(L2)$ is chosen such as to yield an affinity of L2 to micelles, $K_{L2}^{m/o} = 0.1$, that allows neither progressive solubilization (cf. Figure 1) nor formation (cf. Figure 2) of ordered domains within the **fom** range. The results are plotted as Figure S2 in the Supporting Information.

Summarizing, we cannot strictly exclude that a detergent may isolate ordered domains without major changes, but this would be the exception rather than the rule and seems rather unlikely. First, the lipid mixture would need to show favorable properties. Second, the detergent would be required to possess very specific properties with respect to at least two independent parameters. If more membrane components are involved than the two considered here, even more parameters would need to fit in order to eliminate detergent effects. Reports[3−5,14] on different model systems have claimed both the presence and the absence of marked changes in ordered domains upon addition of certain detergents. Even if there is, by chance, an appropriate detergent for a given membrane and temperature, there remains the problem of its identification. As detergent-induced effects may either enhance or diminish ordered domains, it is not justified to assume that the detergent yielding the largest DRM fraction is the correct one. In any case, it is obvious that different detergents will yield different DRMs from the same rafts.

Conclusions

Our calculations yield a number of useful rules:

(1) Thermodynamically, resistance of a lipid against solubilization by a detergent depends only on the affinity of this lipid to the micellar phase, which is quantified by the solubilization boundary of the pure lipid, X_e^{sol}. The presence of another, more susceptible lipid plays no role.

(2) Preferential solubilization of a fluid lipid from mixed membranes increases the relative concentration of the order-preferring lipid there, which may give rise to the growth or appearance of ordered domains.

(3) Detergent-induced formation of ordered domains before the onset of solubilization cannot be explained on the basis of ideal mixing but may result from unfavorable interactions between detergent and order-preferring lipid in the fluid domains.

(4) Thermodynamic (or kinetic) resistance against the detergent is a necessary but not a sufficient criterion for obtaining DRMs. Another key parameter is the size of the resistant membrane fragments. Thermodynamically resistant particles may be so small that they are technically soluble. Cholesterol might (among many other effects) increase the size of resistant particles, thus making them extractable by centrifugation.

(5) Anomalously low resistance of lipids somewhat below their melting temperature could be explained by the accumulation of detergent within minor, fluidlike defect structures between crystal-like gel clusters.

Acknowledgment. We are indebted to Profs. Félix Goñi and Erwin London for their important and constructive comments on the manuscript. This work was supported by the European Commission (Grant No. QLK3-CT-2002-01989 to S.K.) and the Swiss National Science Foundation (Grant No. 31-67216 to H.H.).

Supporting Information Available: Additional results and derivations. This material is available free of charge via the Internet at http://pubs.acs.org.

JA052764Q