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Detergent solubilisation of phospholipid bilayers in the gel state: the role of polar and hydrophobic forces

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Abstract

Testing the solubilisation of phosphatidylcholine (PC) bilayers by Triton X-100 reveals that in the gel state, but not in the fluid state, the amount of detergent required to solubilise the phospholipid is highly dependent on the chain length. Saturated C16 and C18 PC are virtually insoluble at 4°C. However, addition of water-soluble reagents that perturb hydrogen bonding, e.g. urea, or of small proportions of non-bilayer lipids, make the bilayers amenable to detergent solubilisation, even at low temperatures. These results are relevant in the explanation of the origin of detergent-resistant membrane fragments as found, e.g. in caveolae or 'rafts'. © 1998 Elsevier Science B.V. All rights reserved.

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1. Introduction

Detergents, or soluble amphiphiles, are widely used tools in membrane biochemistry. In particular, the solubilisation and reconstitution of integral membrane proteins require the use of these amphiphiles. In recent years, the observation has been made that non-ionic detergents at low (≈4°C) temperatures were unable to solubilise certain membrane fragments, rich in sphingolipids and cholesterol, and often associated with glycosylphosphatidylinositol (GPI)-anchored proteins [1–4]. Schroeder et al. [2] found that lipids with a low T_c gel-to-liquid crystalline phase transition temperature were extracted by cold Triton X-100, while high-T_c phospholipids were not. More recently, Ahmed et al. [3] have associated the non-extractability with the formation of sphingomyelin-cholesterol liquid ordered phases.

These results have stimulated an interest for the interaction of non-ionic detergents with lipids in ordered phases. For the sake of simplicity, we have started by studying the gel ordered L_β phase, that can easily be obtained in single-component bilayers at convenient temperatures, e.g. dipalmitoylphosphatidylcholine below ≈35°C [5]. Specifically, a number of phosphatidylcholines with two identical fatty acyl chains between C14 and C18 have been considered. Large unilamellar vesicles (LUV) have been treated with the commonly used non-ionic detergent Triton X-100, following our previous protocol [6], in which solubilisation is followed through a decrease in turbidity of the liposomal suspension.

Our results indicate that, unlike in the fluid state, solubilisation of phospholipid bilayers well below the T_c transition temperature is highly dependent on the

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chain length. Moreover, studies in which either polar or non-polar interactions in the bilayer had been perturbed by the presence of certain reagents, reveal that both kinds of interactions contribute, in an additive way, to the non-extractability of these bilayers by Triton X-100 at low temperatures.

2. Materials and methods

Triton X-100 (regular, batch 106H06191) was purchased from Sigma (St. Louis, MO). Egg-yolk phosphatidylcholine (PC) was grade I from Lipid Products (South Nutfield, UK). The phospholipids, dioleoyl, dimyristoyl, dipentadecanoyl, dipalmitoyl and distearoylphosphatidylcholine (DOPC, DMPC, DPdPC, DPPC and DSPC, respectively) were from Avanti Polar Lipids (Alabaster, AL). Diacylglycerol (prepared from egg yolk PC) was from Lipid Products; cholesterol, palmitoylcarnitine, and hexadecane were from Sigma. All other reagents were of analytical grade.

The lipids were dissolved in chloroform, mixed as required, and the solvent evaporated exhaustively. Large unilamellar vesicles were prepared by the extrusion method with filters 0.1 μm in diameter [7]. The average diameter of the vesicles was of ca. 100 nm. Lipids were hydrated in 50 mM Tris-HCl, pH 7.0 buffer. Liposome suspensions were mixed with the same volumes of the appropriate detergent solutions, in the same buffer. Final lipid concentration was always 1 mM. Both liposomes and detergent had been previously equilibrated at the desired temperature. The mixtures were left to equilibrate for 1 h at the appropriate temperature, and solubilisation was assessed from the changes in turbidity [8].

Turbidity was measured as absorbance at 500 nm in a Cary Bio 3 spectrophotometer, equipped with thermoregulated cell holders. Turbidity values were normalised by setting 100% as the turbidity of the LUV suspension, 1 mM in lipid, in the absence of detergent, while 0% turbidity corresponded to pure buffer. Under these conditions, D_{50} corresponds to the total detergent concentration producing a 50% decrease in suspension turbidity. This value is obtained from a plot of suspension turbidity vs. detergent concentration. Total, rather than 'effective' [9] Triton X-100 concentrations have been used for convenience. This simplification is acceptable because lipid concentration is kept constant at 1 mM in all measurements. Data for effective Triton X-100 ratios in the solubilisation of PC vesicles have been published elsewhere [10].

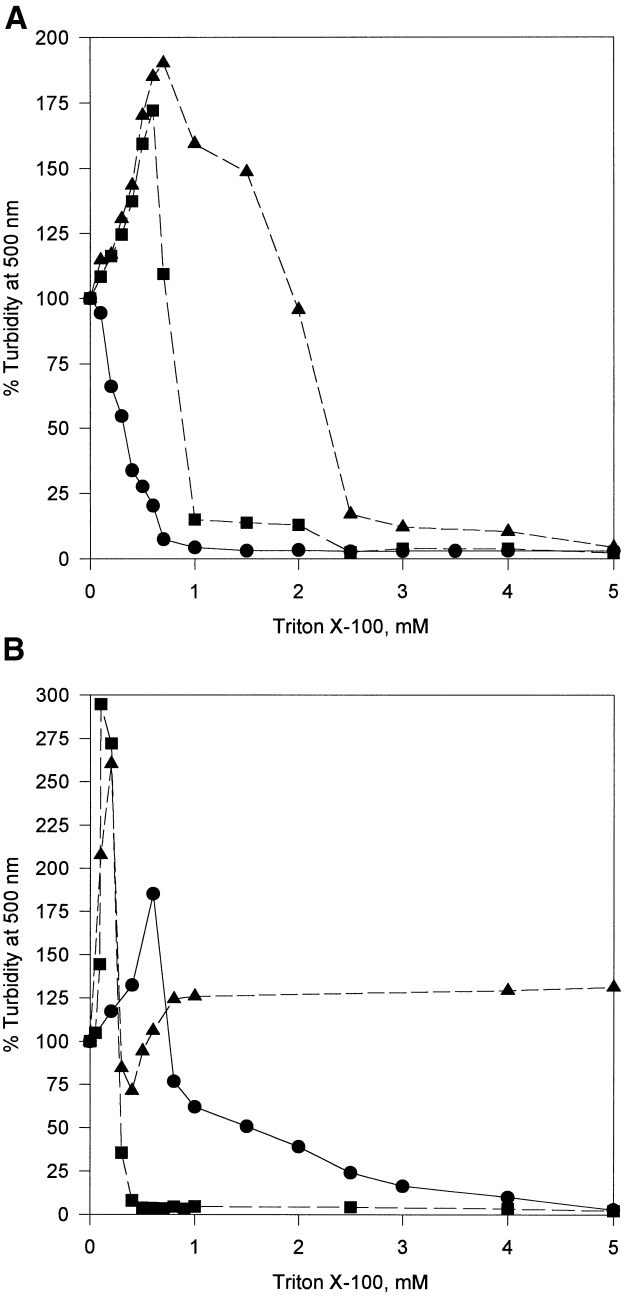

Fig. 1. Detergent solubilisation of phosphatidylcholines. Solubilisation is assayed as a decrease in turbidity of the phospholipid vesicle suspensions. (A) Effect of temperature on DMPC solubilisation: ●, 4°C; ■, 30°C; ▲, 47°C. (B) Effect of chain length, measurements at 37°C: ●, DMPC; ■, DPPC; ▲, DSPC.

3. Results

Representative examples of the solubilisation of phospholipid bilayers at different temperatures are shown in Fig. 1A for the case of DMPC, whose T_c transition temperature is 23°C [5]. At 4°C, i.e. in the L_β gel state, turbidity decreases monotonically with increasing detergent concentrations, while above T_c turbidity first increases, due to a process of vesicle lysis and reassembly, and then decreases, as we had shown a long time ago [8]. Fig. 1A also shows that DMPC solubilisation requires more detergent as temperature is raised. The numerical values for these and other relevant data are given in Table 1.

When similar experiments are performed at different temperatures and with different lipids (Table 1 and Fig. 1B) the resulting picture is more complicated. For example, from solubilisation data at 37°C, DPPC ($T_c \approx 41$°C) appears to be more easily solubilised than DMPC ($T_c \approx 23$°C), but DSPC ($T_c \approx 55$°C) requires even more detergent than DMPC (Table 1).

The above results can be more easily understood by plotting the different solubilisation data as a function of 'corrected temperatures', i.e. temperatures expressed in °C above (+) or below (−) the T_c transition temperature of each lipid. For example the 'corrected temperature = +10°C' will correspond to +51°C for DPPC and +33°C for DMPC. When this correction is made, the plot shown in Fig. 2 is obtained. Two situations are clearly distinguished, above and below T_c. Above (or near) T_c, i.e. in the region of corrected temperatures near or above zero, the various lipids behave similarly, and the respective solubilisation data overlap in the plot shown in Fig. 2. However, well below T_c this is not the case, solubilisation requires more Triton X-100 the longer the phospholipid acyl chain. Examination of the D_{50} data in Fig. 3 at the corrected temperatures of −4 and −20°C illustrates this point very clearly. The minimum D_{50} values in Fig. 2 are not seen precisely at the T_m gel–fluid transition temperature of the pure phospholipid (corrected $T = 0$°C), but at somewhat lower temperatures. This may be due to the fact that Triton X-100 tends to decrease T_c of the mix-

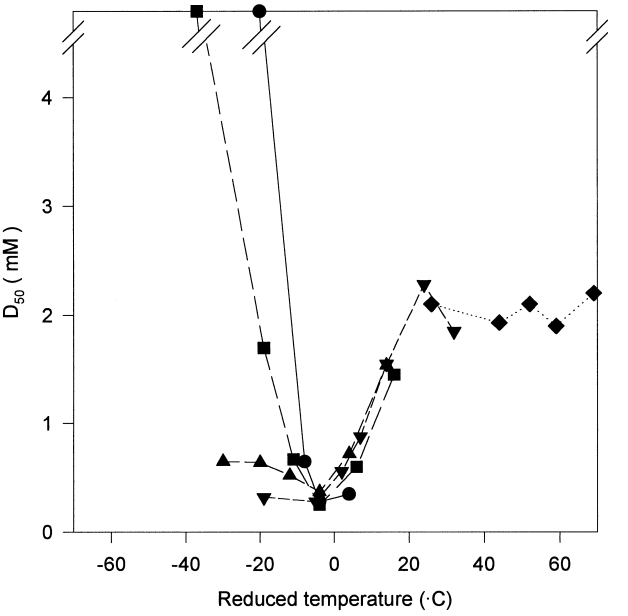

Fig. 2. Triton X-100 solubilisation of phosphatidylcholines as a function of 'corrected temperature'. Corrected temperature is defined as temperature (in °C) above or below the T_c gel-to-liquid crystalline transition of the phospholipid. Total detergent concentrations producing 50% solubilisation are plotted versus corrected temperatures. ◆, DOPC; ▼, DMPC; ▲, DPdPC; ■, DPPC; ●, DSPC.

Table 1
Detergent (Triton X-100) concentration (mM) for 50% solubilisation of different phospholipid-LUVs at different temperatures

LUV composition	Temperature (°C)							
	4	20	30	37	47	51	55	59
EYPC	1.85	1.90	2.15	2.08	2.25	–	–	–
DOPC	2.10	1.93	2.10	1.90	2.20	–	–	–
DMPC	0.32	0.28	0.88	1.55	2.28	–	1.75	–
DPdPC	0.65	0.52	0.37	0.72	1.54	–	–	–
DPPC	> 5.0	1.7	0.67	0.25	0.60	–	1.45	–
DSPC	> 5.0	> 5.0	> 5.0	> 5.0	0.68	0.28	–	0.35

Data are derived from turbidity vs. detergent concentration plots as shown in Fig. 1. Final phospholipid composition was 1 mM in all cases.

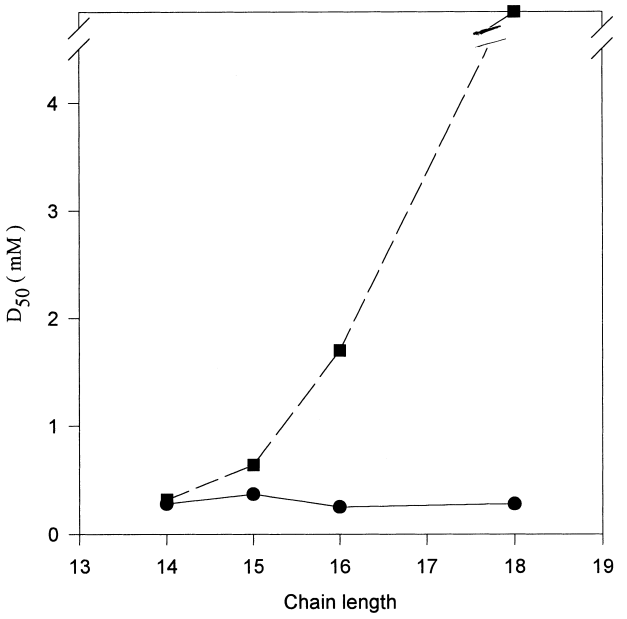

Fig. 3. Effect of phospholipid fatty acyl chain length on the amount of detergent required for bilayer solubilisation. The total Triton X-100 concentration reducing by 50% the original liposomal suspension turbidity (D_{50}) is plotted versus fatty acyl chain length of DMPC, DPdPC, DPPC and DSPC at the corrected temperatures −4°C (●) and −20°C (■).

tures [17]. Thus solubilisation would be most favoured at the transition temperature of the phospholipid–detergent mixture.

In summary, our studies on the solubilisation of phospholipids in the gel state reveal that: (a) for a given phospholipid below T_c, solubilisation requires more detergent the lower the temperature; and (b) at a given temperature below T_c, solubilisation requires more detergent the longer the acyl chain. In order to explain these phenomena, we propose a hypothesis, and describe some experimental tests for it. According to our hypothesis, detergent–phospholipid interaction, an essential step in solubilisation [11] is made difficult by the highly ordered, quasi-crystalline state that occurs in the bilayer gel phase. Phospholipid bilayers are stabilised in aqueous media by hydrophobic chain–chain interactions as well as by the polar interactions of the phospholipid headgroups between them and with water [12]. In the various phospholipids under study, the headgroup was always phosphorylcholine, thus polar interactions must be essentially similar in all cases. There were, however, significant differences in the fatty acyl chains. Some were unsaturated, as egg PC or POPC, and the *cis*-unsaturation prevented their going into the gel phase in the temperature range of our study. Others were fully saturated, and in those cases the longer the hydrophobic chain the stronger the hydrophobic stability component.

According to this hypothesis, any element that tends to perturb the quasi-crystalline arrangement of phospholipids in the gel phase, by modifying either the polar or the hydrophobic interactions in the bilayer, is likely to make the membrane more easily amenable to detergent solubilisation. Our experimental approach is based on modifying the intermolecular interactions of DSPC at 37°C. Under these conditions, even the higher detergent concentrations tested (5 mM) were far from producing any significant solubilisation (Table 1, Fig. 1B).

First, polar interactions, and in particular hydrogen-bonding of water to phospholipid polar headgroups were perturbed by addition of chaotropic agents. Representative examples of the effects of urea (3 M), guanidinium chloride (2 M) or ammonium sulphate (4%) are shown in Fig. 4A. In all cases, DSPC becomes to some extent solubilised by Triton X-100 at 37°C, which did not happen in the absence of these reagents, the corresponding D_{50} values being summarised in Table 2. In these experiments, the chaotropic agents are added in concentrated solutions to the preformed liposomes, and the detergent is also equilibrated with the chaotropic agent. One hundred percent turbidity corresponds to the LUV suspension in the presence of the chaotropic agent. Control experiments have recently confirmed [13] that addition of 3 M urea does not produce major changes in the bilayer architecture or vesicle size. Specifically, quasi-elastic light scattering

Table 2
Detergent concentrations (mM) for 50% solubilisation of DSPC-LUVs in the presence of various additives at 37°C

DSPC (control)	≫5.00
+3.0 M urea	3.56
+2.0 M guanidinium HCl	0.36
+4% $(NH_4)_2$ SO_4	2.30
+3% cholesterol	5.00
+3% palmitoylcarnitine	2.90
+3% diacylglycerol	3.00
+3% lyso PC	2.50
+3% hexadecane	3.35
+3% hexadecane+3.0 M urea	0.34

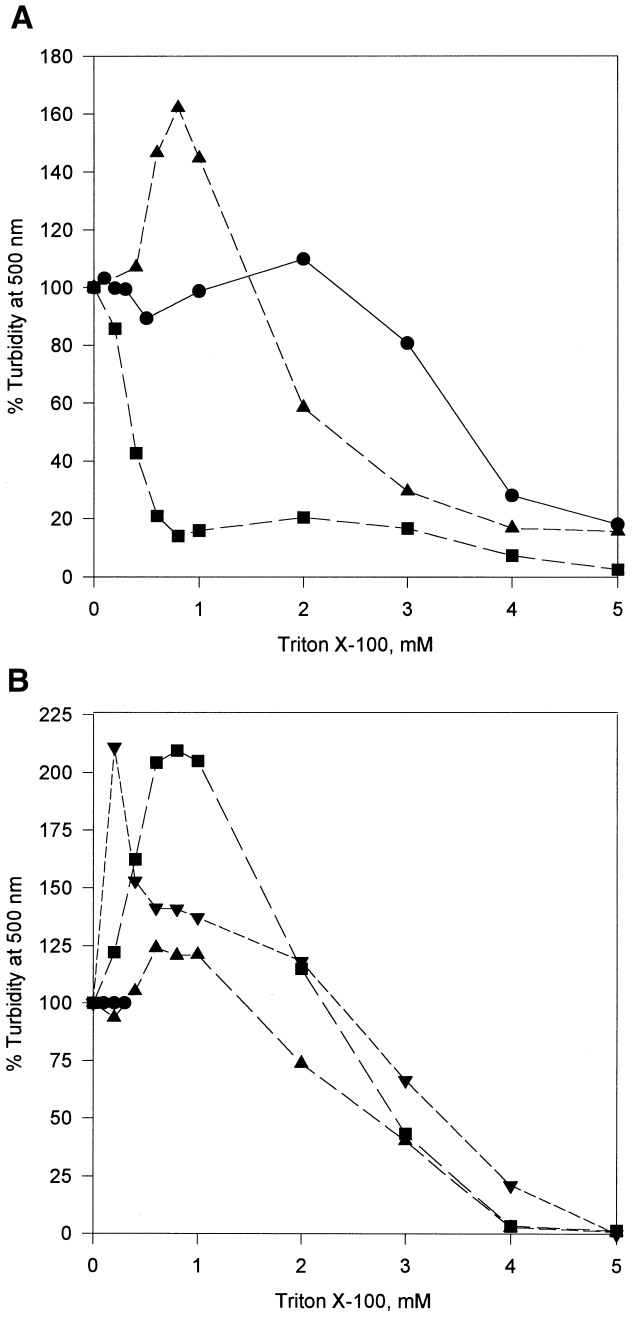

Fig. 4. Effect of various additives on Triton X-100 solubilisation of DSPC bilayers at 37°C. (A) Water-soluble additives: ●, 3 M urea; ■, 2 M guanidinium chloride; ▲, 4% ammonium sulphate. (B) Lipid-soluble additives, all at 3 mol%: ■, palmitoylcarnitine; ▼, hexadecane; ▲, lysopalmitoylphosphatidylcholine.

measurements of vesicle size showed an average increase of 12% in vesicle diameter after external addition of urea to a 3 M concentration.

Other experiments were directed to perturbing mainly the non-polar region of the phospholipid bilayer. For that purpose, a number of lipids (cholesterol, diacylglycerol, palmitoylcarnitine, lysophosphatidylcholine or hexadecane) were mixed at 3 mol% with DSPC in organic solvent prior to liposome preparation. Many data in the literature confirm that these lipids are readily incorporated into the bilayer under those conditions, although in some cases (particularly with lysophosphatidylcholine) a fraction of the amphiphile may remain in the water phase. Some of these lipids, e.g. hexadecane will almost exclusively alter the hydrophobic matrix, while others are likely to influence both the polar and non-polar regions of the bilayers, e.g. palmitoylcarnitine. In any case, all of them happen to increase considerably the solubilisation of DSPC by Triton X-100 (Fig. 4B and Table 2).

The effects of water-soluble and lipid-soluble perturbing agents are additive, as shown by e.g. the effect of 3 M urea when added to preformed DSPC liposomes containing 3% hexadecane. In this case, 50% solubilisation is observed with just 0.34 mM Triton X-100 (Table 2), a detergent concentration comparable to the one producing 50% solubilisation of DSPC at or near T_c (Table 1).

4. Discussion

The two main observations reported in this paper are: (a) that C16 and C18 saturated phosphatidylcholines in the gel phase are solubilised by Triton X-100 with much more difficulty than when they are in the fluid phase: and (b) that the difficulty may be reverted by perturbing either or both the polar and non-polar interactions whose equilibrium leads to membrane stability.

The decrease in detergent solubilisation when the lipid goes below T_c had been the object of an early study by nuclear magnetic resonance [14]. In a previous study by our group, DMPC solubilisation by Triton X-100 was carefully tested by a filtration procedure at 4 and 37°C, and found to be invariant with temperature [15]. The latter results are not in agreement with the data in this paper, yet they are probably correct. The difference in the method for assessing solubilisation, that included in [15] passing the

detergent-treated suspension through a narrow-pore filter, under pressure, may explain the different results, since the filtration shear is probably potentiating the disruptive effects of Triton X-100.

Helenius and Simons [16] suggested that the first step in membrane solubilisation should be the insertion of monomeric detergent molecules into the bilayer. This prelytic binding has been quantitated for the case of Triton X-100 and fluid phosphatidylcholine bilayers [17]. It is understandable that detergent insertion is more difficult in an ordered bilayer. In fact, in the quasi-crystalline purple membrane Triton X-100 is virtually unable to become inserted among the phospholipids [18]. In the cases described in the present paper, solubilisation requires higher detergent concentrations particularly for long-chain (C16 and C18) phosphatidylcholines well below their T_c. This is probably due to the fact that these bilayers exhibit, apart from the large increase in order when cooling through T_c, a smaller but progressive ordering, once in the gel state, with decreasing temperatures [19]. Note that, even when solubilisation does not occur (e.g. Fig. 1B, DSPC at 37°C) some detergent-binding takes place, judging from the increase in suspension turbidity at prelytic detergent concentrations (see [8] for the phenomenon of increased turbidity).

Hydrophobic interactions are essential for membrane structure [20], while the role of lateral polar interactions between glycosphingolipids in the formation of certain membrane microdomains has been recently emphasised [21,22]. The presence of reagents that perturb either the polar or the hydrophobic interactions in the bilayer is likely to induce the molecular fluctuations or transient packing defects that will allow detergent binding and subsequent solubilisation. When the perturbing agents are lipids, a small proportion (3 mol%) is enough to disrupt the quasi-crystalline structure and allow the entrance of detergent molecules. In a different context, 3 mol% cholesterol in DMPC has been shown to have a considerable effect in the binding of the *Escherichia coli* toxin α-haemolysin [23]. The essentially different nature of the polar and hydrophobic interactions stabilizing the bilayer structure explains the additive effect of urea and hexadecane (Table 2). While this combined effect is conceptually interesting, the effect of the water-soluble reagents by themselves (urea, at concentrations that do not produce irreversible protein denaturation, guanidinium chloride, ammonium sulphate) (Table 2 and Fig. 3A) may be important in solubilising detergent-resistant membrane fractions, thus in the purification of certain GPI-bound proteins [1–4].

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