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Detergent solubilization of phosphatidylcholine bilayers in the fluid state: Influence of the acyl chain structure

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Abstract

Seventeen different, chemically defined phosphatidylcholines, dispersed in aqueous medium in the form of large unilamellar vesicles, have been tested for solubilization by the non-ionic detergent Triton X-100. The temperatures (either 20 °C or 45 °C) were such that the bilayers were always in the liquid-disordered state. For each case, the solubilization parameters, D_{on} (total detergent: lipid mole ratio producing the onset of solubilization) and D_{50} (total detergent: lipid mole ratio producing 50% solubilization), were determined under equilibrium conditions. Both parameters varied generally in parallel. When double bonds were introduced to the acyl chains, other factors remaining constant, solubilization became more difficult, i.e., more detergent was required. *Cis*-unsaturated phospholipids required more detergent than the corresponding *trans*-isomers. Increasing chain length in saturated phospholipids between C12 and C16 decreased moderately the detergent/lipid ratios causing solubilization. Acyl and alkyl phospholipids were equally susceptible to Triton X-100 solubilization. Lipid chain order, as measured by DPH fluorescence polarization, seemed to facilitate solubilization, perhaps because more ordered bilayers have a smaller capacity to accommodate detergent monomers without breaking down into lipid–detergent mixed micelles.

Keywords: Membrane solubilization; Detergent; Surfactant; Lipid phase; Lipid order

1. Introduction

Detergents are important tools in the study of biomembranes. In particular, they are essential in the solubilization and reconstitution of integral membrane proteins (see [1] for a collection of reviews on detergents). According to the well-known three-stage model of Helenius and Simons [2], detergent monomers insert into the membrane bilayer (stage I), then the bilayer becomes saturated with detergent, and lipid–detergent (or protein–lipid–detergent) mixed micelles start to form (stage II), finally the mixed micelles become further enriched in detergent (stage III). Previous studies from this laboratory have characterized in detail the various stages of PC bilayer solubilization by Triton X-100 [3]. The comparative study of several members of the Triton X series, containing the same hydrophobic moiety but polar groups of different lengths, showed an optimum hydrophile/lipophile balance of 15, corresponding to Triton X-102 [4]. Conversely, a study in which the hydrophobic chain length of different electrically charged detergents was systematically varied demonstrated that the detergent concentration required to produce the onset of solubilization (D_{on}) decreased as the hydrophobic chain length increased [5].

Abbreviations: D_{on}, total detergent: lipid mole ratio producing the onset of solubilization; D_{50}, total detergent: lipid mole ratio causing 50% solubilization; DPH, diphenylhexatriene; LUV, large unilamellar vesicles; DAPC, diarachidonoyl phosphatidylcholine; DEPC, dielaidoylphosphatidylcholine; DLaPC, dilauroyl phosphatidylcholine; DLPC, dilinoleoyl phosphatidylcholine; DLnPC, dilinolenoyl phosphatidylcholine; DMPC, dimyristoyl phosphatidylcholine; DMoPC, dimyristoleoyl phosphatidylcholine; DOPC, dioleoyl phosphatidylcholine; DPPC, dipalmitoyl phosphatidylcholine; DPePC, dipentadecanoyl phosphatidylcholine; DPoPC, dipalmitoleoyl phosphatidylcholine; DPhPC, diphytanoyl phosphatidylcholine; DPsPC, dipetroselenoyl phosphatidylcholine; DTPC, ditridecanoyl phosphatidylcholine; HAPC, 1-hexadecyl-2-arachidonoyl phosphatidylcholine; PAPC, 1-palmitoyl-2-arachidonoyl phosphatidylcholine; PC, phosphatidylcholine; POPC, 1-palmitoyl-2-oleoyl phosphatidylcholine

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In the recent years, detergents have attracted a renewed attention from the scientists, as a result of the observation that at least some membrane microdomains ("rafts") may be relatively resistant to detergent solubilization at low temperature (see review in [6]). This has prompted a large number of studies both from the cell biology and the biophysical points of view. Among the latter, Lichtenberg and co-workers have studied in detail the effect of temperature on detergent solubilization [7,8] concluding that increasing temperature tends in general to augment the amount of detergent required for membrane solubilization. Heerklotz and co-workers [9,10] have carried out detailed calorimetric measurements of detergent–membrane interaction, and have demonstrated that some detergents, including Triton X-100, may give rise to artefactual "detergent-resistant domains" in some lipid bilayers. Relevant studies from this laboratory include the effect of phospholipid acyl chain length on the solubilization of bilayers in the gel state [11], in which it was found that the amount of Triton X-100 required for solubilization increased enormously with chain length under those conditions. It was also found that bilayers in the liquid-ordered state were less susceptible to solubilization than those in the liquid-disordered, or fluid, state [12].

In the present study, we have performed an extensive survey on the influence of the fatty acyl structure on the solubilization properties of phosphatidylcholines (PC) in the fluid state, i.e., the physical state in which most cell membrane lipids are considered to exist. A wide variety of chemically defined PC's have been used to prepare bilayers, in the form of large unilamellar vesicles (LUV), and the resulting vesicles have been treated with increasing concentrations of Triton X-100. Solubilization has been assessed as a decrease in suspension turbidity. We have found that the main single structural factor influencing solubilization is the presence of double bonds in the acyl chains, unsaturation increasing the amount of detergent required for solubilization. In general, increasing molecular order in the bilayers (within the fluid, or liquid-disordered state) facilitates solubilization by Triton X-100.

2. Materials and methods

All phospholipids were supplied by Avanti Polar Lipids (Alabaster, AL). Triton X-100 (batch no. 125H0569) was purchased from Sigma (St. Louis, MO). DPH was from Molecular Probes (Alabaster, AL). All lipids were used without further purification. All other reagents were of analytical grade.

The lipids were dissolved in chloroform :methanol (2:1,v/v) and mixed as required, and the solvent was evaporated exhaustively. Multilamellar vesicles (MLV) were prepared by hydrating the dry lipids in buffer, with vortex shaking. Lipids were hydrated in excess 10 mM HEPES, 150 mM NaCl, pH 7.4. Large unilamellar vesicles were prepared by the extrusion method (10 passages) with filters 0.1 μm in pore diameter [13]. Vesicle size was measured by quasi-elastic light scattering in a Malvern Zeta-Sizer 4 spectrometer. The average diameter of the vesicles was in all cases ca. 100 nm.

2.1. Solubilization assays

Liposome suspensions were mixed with the same volumes of the appropriate Triton solutions in the same buffer. Final lipid concentration, measured as lipid phosphorus, was 1 mM. The mixtures were left to equilibrate for 1 h at desired temperature, and solubilization was assessed from the changes in turbidity [14]. Preliminary experiments had shown that 1 h was enough to reach an apparent equilibrium in the LUV-detergent systems. Turbidity was measured as absorbance at 500 nm in a UVIKON spectrophotometer, with continuous sample stirring. Turbidity values were normalized by setting 100% as the turbidity of the LUV suspension, 1 mM in lipid, in the absence of Triton, while 0% turbidity corresponded to pure buffer. The absolute value of turbidity (A_{500}) for the 1 mM LUV suspensions in the absence of detergent was 0.03–0.04, irrespective of lipid composition and temperature, in the 20–45 °C range, for lipids in the fluid state.

2.2. Fluorescence polarization assays

The fluorescence polarization of DPH was measured using a SLM 8100 Spectrofluorometer, equipped with standard polarization accessories and a circulating water bath. The excitation and emission wavelengths were 360 and 430 nm, respectively.

The fluorescence polarization was calculated as:

$$P = (I_{vv} - GI_{vh})/(I_{vv} + GI_{vh})$$

where I_{vv} and I_{vh} represent the intensity of vertically and horizontally polarized fluorescence light when excitation light is vertically polarized. The correction factor $G = I_{hv}/I_{hh}$. I_{hv} and I_{hh} represent the intensity of vertically and horizontally polarized fluorescence light when excitation light is horizontally polarized. The fluorescent probe DPH was added to the phospholipid in organic solution to obtain a probe/lipid molar ratio of 1/250.

3. Results and discussion

Solubilization of phosphatidylcholine bilayers by Triton X-100 was studied through changes in turbidity (A_{500}) of pure phospholipid membranes in the form of LUV, incubated with increasing concentrations of detergent. For each experiment, two parameters were evaluated, respectively D_{on}, or the detergent concentration causing detergent saturation of the phospholipid bilayer at the onset of solubilization [15], and D_{50}, or the detergent concentration causing a 50% decrease in turbidity of the vesicle suspension [11]. Fig. 1 shows a representative solubilization experiment using diphytanoyl PC vesicles, and the graphical determination of D_{on} and D_{50}. The numerical values can be found in Table 1, #1. D_{sol}, or the detergent concentration causing full bilayer solubilization (<10% of the original turbidity) could in principle be a useful

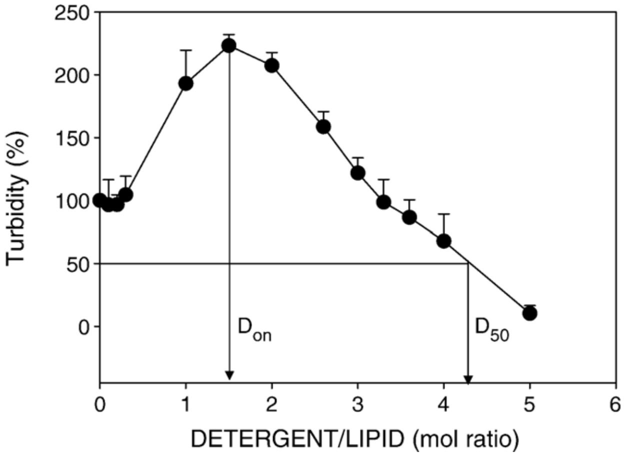

Fig. 1. Solubilization of LUV composed of DPhPC was assessed by decrease in turbidity as a function of Triton X-100 concentration. The graphical determination of the solubilization parameters D_{on} and D_{50} is shown. The experimental points correspond to average values ± S.E.M. (n=3). T=20 °C.

Table 1
Solubilization parameters of phosphatidylcholine bilayers in the presence of Triton X-100

#	Phospholipid	Fatty acid structure	T_c (°C) [a]	T (°C) [b]	D_{on}	D_{50}
1	DPhPC	Phytanoyl	≪0	20	1.5±0.0	4.2±0.07
Effect of chain length						
2	DLaPC	C12:0	0	45	2.6±0.04	4.8±0.01
3	DTPC	C13:0	14	45	2.3±0.0	3.8±0.0
4	DMPC	C14:0	23	45	2.3±0.0	3.8±0.0
5	DPePC	C15:0	34	45	2.0±0.0	3.6±0.0
6	DPPC	C16:0	41	45	2.3±0.07	3.6±0.14
7	DMoPC	C14:1	−4	45	3.0±0.0	4.8±0.02
8	DPoPC	C16:1	−36	45	3.0±0.07	4.8±0.07
9	DOPC	$C18:1^{9}$	−20	45	3.6±0.0	4.5±0.07
Effect of chain unsaturation						
10	DMPC	C14:0	23	45	2.3±0.0	3.8±0.0
11	DMoPC	C14:1	−4	45	3.0±0.0	4.8±0.02
12	DPPC	C16:0	41	45	2.3±0.07	3.6±0.14
13	DPoPC	C16:1	−36	45	3.0±0.07	4.8±0.07
14	DOPC	$C18:1^{9}$	−20	20	1.25±0.35	3.9±0.0
15	DLPC	$C18:2^{9.12}$	−53	20	1.5±0.0	4.0±0.0
16	DLnPC	$C18:3^{9.12.15}$	−63	20	1.5±0.0	4.8±0.0
17	POPC	C16:0, $C18:1^{9}$	−2	20	1.0±0.0	3.8±0.07
18	DOPC	$C18:1^{9}$	−20	20	1.25±0.35	3.9±0.0
19	PAPC	C16:0, C20:4	−20	20	1.0±0.0	3.1±0.0
20	DAPC	C20:4	−70	20	2.0±0.0	5.0±0.0
Miscellaneous factors						
21	DOPC	$C18:1^{9}$	−20	20	1.25±0.35	3.9±0.0
22	DPsPC	$C18:1^{6}$	1	20	1.25±0.35	3.0±0.05
23	DOPC	$C18:1^{9}$ cis	−20	45	3.6±0.0	4.5±0.07
24	DEPC	$C18:1^{9}$ trans	11	45	1.5±0.0	2.5±0.05
25	PAPC	C16:0, C20:4	−20	20	1.0±0.0	3.1±0.0
26	HAPC	C16:0 (ether), C20:4	−20	20	1.0±0.0	3.0±0.05

Phospholipids were dispersed in buffer in the form of LUV. Solubilization was assessed as a decrease in turbidity. Data derived from experiments as shown in Fig. 1. Average values of three independent experiments ± S.E.M.

[a] T_c is the gel-to-liquid crystalline transition temperature of the fully hydrated phospholipid. Data taken from http://www.lipidat.chemistry.ohio-state.edu.

[b] T is the actual temperature at which the solubilization experiment was performed.

parameter, but in some cases full solubilization is not achieved even at the highest detergent: lipid mole ratio tested (5:1), thus D_{sol} was not computed. Note, however, that at 20:1 detergent: lipid ratios all lipids tested in this work were fully solubilized (data not shown)].

For virtually all compositions under study, low detergent concentrations cause an *increase* in turbidity. This is due to detergent-induced lysis and reassembly ("fusion") of the vesicles [16,17]. As observed previously [16] this phenomenon occurs only when bilayers are in the fluid state and, as will be seen below, larger increases in turbidity are usually observed for the lipids with lower gel–fluid transition temperatures (see T_c data in Table 1). For the sake of simplicity, all experiments reported here have been performed at a single lipid concentration of 1 mM. Strictly speaking, D_{on} results cannot be compared with each other, since the detergent partition coefficient into the different bilayers need not be the same. A precise comparison would require the use of Re^{sat}, or the *effective* detergent/lipid ratio in the bilayers [15], at the onset of solubilization. This parameter is derived from solubilization experiments at varying lipid concentrations [15]. However, the available data of Re^{sat} for Triton X-100 and different lipids indicate that D_{on} values obtained under our conditions can be used as a good approximation to Re^{sat} [14,15].

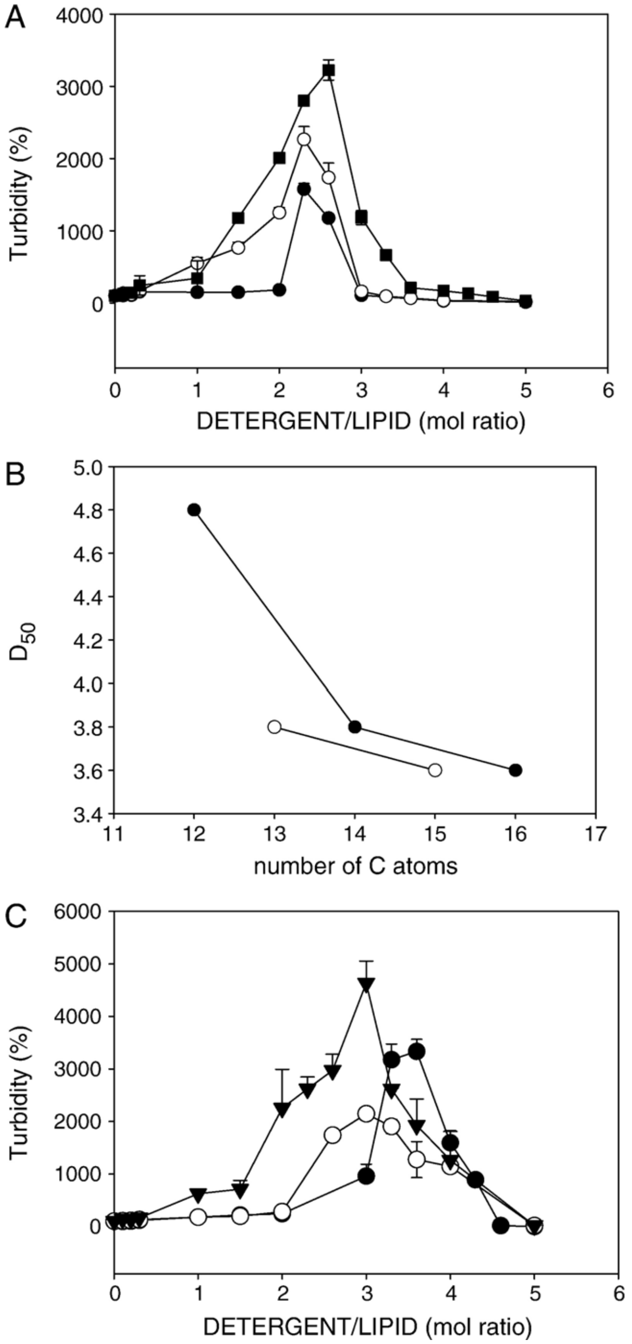

Fig. 2. Effect of chain length on the solubilization of LUV by Triton X-100. (A) Saturated chains. (■) DLaPC. (○) DMPC. (●) DPPC. (B) Saturated chains. The change in D_{50} as a function of acyl chain length. (●) Even numbers. (○) Odd numbers. (C) Unsaturated chains. (●) DOPC. (○) DPoPC. (▼) DMoPC. The experimental points correspond to average values ± S.E.M. ($n = 3$). $T = 45$ °C.

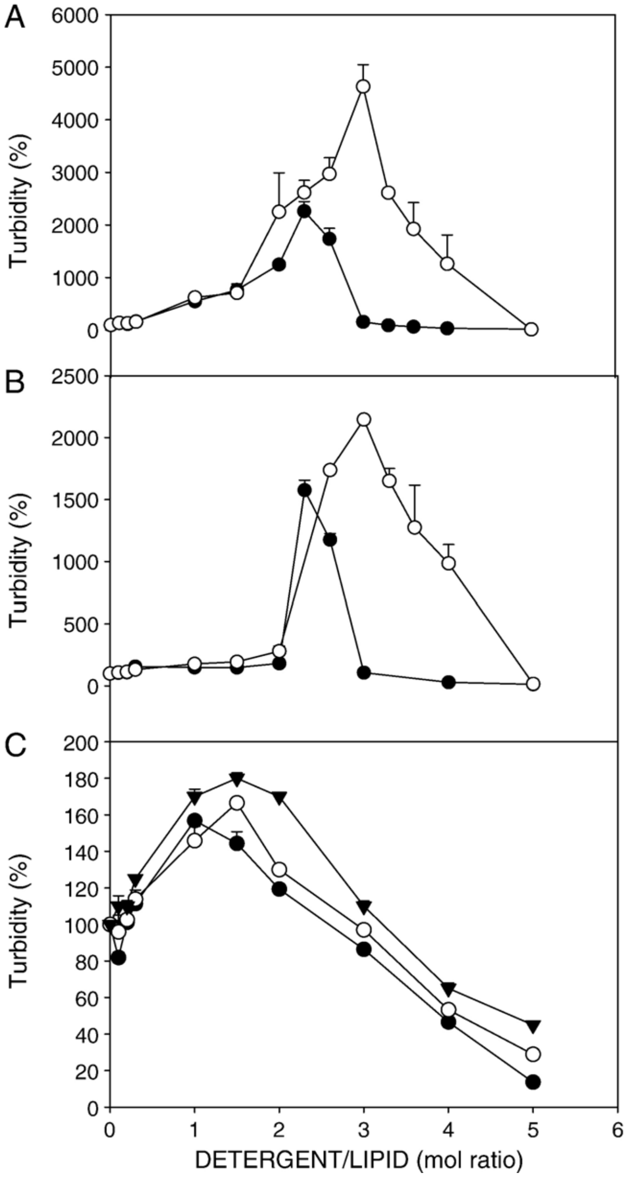

Fig. 3. Effect of fatty acyl chain unsaturation on the solubilization of LUV by Triton X-100. (A) C14 chains. T=45 °C. (○) DMoPC. (●) DMPC. (B) C16 chains. T=45 °C. (○) DPoPC. (●) DPPC. (C) Unsaturated C18 chains. T=20 °C. (●) DOPC. (○) DLPC. (▼) DLnPC. The experimental points correspond to average values±S.E.M. (n=3).

3.1. Effect of chain length

The effect of chain length on detergent solubilization was tested using two series of homologous PC's, respectively saturated and monounsaturated. The saturated series included DLaPC, DTPC, DMPC, DPePC and DPPC, and the monounsaturated series was formed by DMoPC, DPoPC and DOPC. The fatty acid structures of these phospholipids are given in an abbreviated form in Table 1. Solubilization was tested at 45 °C, a temperature at which all eight lipids are in the fluid state.

The results for the saturated lipids at 45 °C are shown in Fig. 2A, B (Table 1, #2–6). Both D_{on} and D_{50} decrease slightly with increasing chain length, the corresponding values being lower for the odd-numbered fatty acyl chains (Fig. 2B). Solubilization of the monounsaturated series DMoPC, DPoPC and DOPC, measured at 45 °C, i.e., well-above the gel–fluid transition temperature of these lipids, is shown in Fig. 2C, and the solubilization parameters are collected in Table 1, #7–9. Both D_{on} and D_{50} remain virtually constant with chain length. Apparently, phospholipid chain length is not a major factor influencing the amount of detergent required to produce solubilization, when the phospholipid bilayers are in the fluid state.

3.2. Effect of chain unsaturation

The effect of introducing one or more double bonds in the fatty acyl chains of PC on solubilization was tested by comparing saturated and monounsaturated couples, such as DMPC and DMoPC (Fig. 3A), or DPPC and DPoPC (Fig. 3B). The mono-, di- and triunsaturated forms of the C18 fatty acid were also tested, as seen in Fig. 3C. Introducing one double bond in the chain, at temperatures at which both phospholipids are in the fluid phase, increases the amount of detergent required for solubilization, by about 1 molecule Triton X-100/PC molecule (Table 1, #10–13). Introducing a second and a third double bond in the fatty acyl chain, as shown for the solubilization of DOPC, DLPC and DLnPC (Fig. 3C) also increases the amount of detergent required for solubilization,

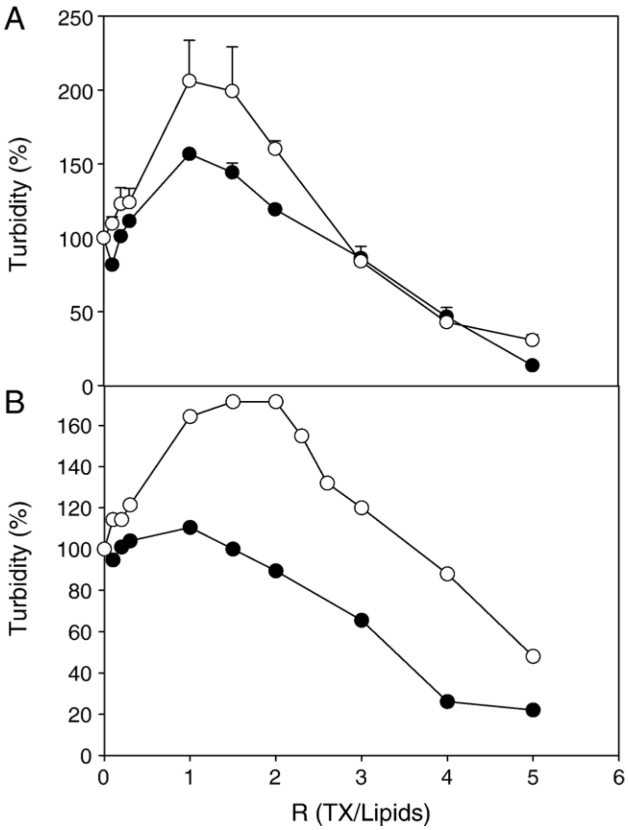

Fig. 4. Effect of one versus two unsaturated fatty acyl chains on the solubilization of LUV by Triton X-100. (A) (○) POPC. (●) DOPC. (B) (●) PAPC. (○) DAPC. The experimental points correspond to average values±S.E.M. (n=3). T=20 °C.

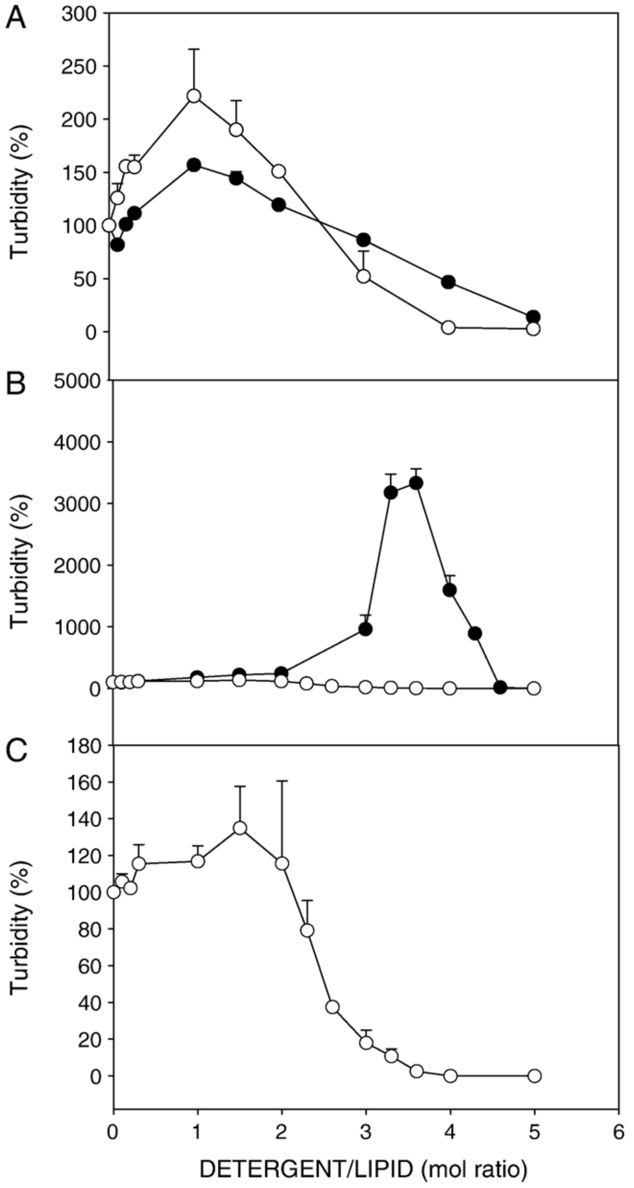

Fig. 5. Further effects of fatty acyl chain unsaturation on the solubilization of LUV by Triton X-100. (A) Effect of double bond position. $T=20$ °C (○) DPsPC. (●) DOPC. (B) Effect of *cis–trans* isomery. $T=45$ °C. (○) DEPC. (●) DOPC. (C) Expanded plot of DEPC solubilization at 45 °C. The experimental points correspond to average values±S.E.M. ($n=3$).

although in this case the effect is quantitatively smaller (Table 1, #14–16). Note that, in this case, no data for the saturated homologue DSPC are available, because DSPC has a gel-to-fluid transition temperature $T_c=55$ °C, so that temperatures at which this phospholipid is in the fluid state are above the so-called "cloud point" of Triton X-100, and this interferes with the turbidity measurements of solubilization.

The effect of two versus one unsaturated chain in the same PC molecule was also examined (Fig. 4). When POPC and DOPC are compared, the difference in detergent requirement is very small (Fig. 4A, and Table 1, #17, 18), but when the difference in unsaturation is larger, e.g., PAPC and DAPC (Fig. 4B, and Table 1, #19, 20), the amounts of detergent required for solubilization differ clearly, showing again that unsaturated phospholipids require as a rule more detergent for solubilization than their saturated counterparts.

3.3. Miscellaneous factors

The effect of other fatty acyl structural factors on the solubilization of PC bilayers by Triton X-100 was equally examined, namely position of the double bond, *cis/trans* isomery, and acyl or alkyl nature of the chain. Oleic acid has a *cis* double bond in C9–10, but the far less common petroselenic acid has a *cis* double bond in C6–7. The latter has a higher melting point than oleic acid [18]. A comparative study of the solubilization of DOPC and DPsPC (Fig. 5A, Table 1, #21, 22) shows that less detergent is required to solubilize the phospholipid containing fatty acids with a higher melting point. This is in line with the previous observation (Fig. 3) that the unsaturated phospholipids are more resistant towards Triton X-100 solubilization than their saturated homologues. The influence of the *cis–trans* isomery was tested, as seen in Fig. 5B, comparing the solubilization of DOPC and DEPC (see also Table 1, #23, 24). As predicted in the light of our previous observations, the *cis*-double bond containing phospholipid DOPC requires for its solubilization more detergent than DEPC, whose physical properties resemble those of the saturated PC's [11].

Finally, the question on whether the alkyl and acyl forms of a phospholipid display or not different solubilization properties was answered, by comparing PAPC and HAPC. Exactly the same solubilization parameters were found (Table 1, #25, 26).

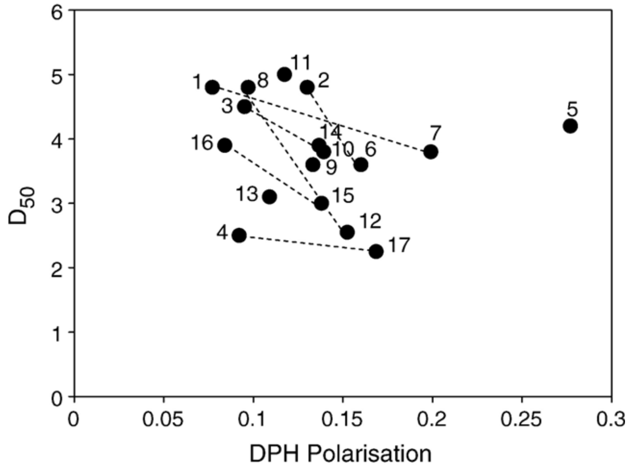

Fig. 6. Effect of bilayer acyl chain order on detergent solubilization. Molecular order has been estimated by measuring polarization of DPH fluorescence. Solubilization has been estimated by the D_{50} parameter (see main text for details). Experimental points correspond to: (1) DMoPC (45 °C); (2) DPoPC (45 °C); (3) DOPC (45 °C); (4) DEPC (45 °C); (5) Diphytanoyl PC (20 °C); (6) DPPC (45 °C); (7) DMPC (45 °C); (8) DLaPC (45 °C), (9) DPePC (45 °C); (10) DTPC (45 °C); (11) DAPC (20 °C); (12) DLaPC (20 °C); (13) PAPC (20 °C); (14) DOPC (20 °C); (15) DPsPC (20 °C), (16) DPsPC (45 °C); (17) DEPC (20 °C). Saturated–unsaturated couples, e.g., DMPC-DMoPC (7–1), or high- and low-temperature couples, e.g., DOPC at 45 °C and 20 °C (3–14), are tied by dotted lines.

Table 2
The effects of temperature on the solubilization of PC bilayers by Triton X-100

Phospholipid	D_{on}		D_{50}	
	20 °C	45 °C	20 °C	45 °C
DLaPC	1.5±0.0	2.6±0.04	2.6±0.0	4.8±0.01
DMPC	1.5±0.0 [a]	2.3±0.0	2.8±0.14 [a]	3.8±0.0
DMoPC	1.0±0.0	3.0±0.0	2.8±0.0	4.8±0.02
DPoPC	2.3±0.05	3.0±0.07	3.5±0.07	4.8±0.07
DOPC	1.25±0.35	3.6±0.0	3.9±0.0	4.5±0.07

Phospholipids dispersed in buffer in the form of LUV. Solubilization assessed as a decrease in turbidity. Data derived from experiments as shown in Fig. 1. Average values of three independent experiments±S.E.M.

3.4. Solubilization and molecular order

In view of the above results showing that unsaturated phospholipids require more detergent for solubilization, a study was performed in which the molecular order (or microviscosity) of selected bilayers was evaluated measuring polarization of DPH fluorescence. This parameter is frequently taken as an estimation of a local "microviscosity" [19], or of molecular order in membranes [20]. When D_{50} is plotted as a function of DPH fluorescence polarization it can be seen that, in general, a negative correlation appears to exist between D_{50} (amount of detergent that causes 50% solubilization) and DPH polarization (or degree of molecular order in the bilayer) (Fig. 6). A very clear relationship is seen when specific saturated–unsaturated couples are compared (tied by lines in Fig. 6). In agreement with the observed correlation between molecular order/microviscosity and D_{50}, both D_{50} and D_{on} appear to be lower for the lipids with higher T_c gel–fluid transition temperatures. (Table 1) because, at a given temperature, order and microviscosity should be higher for the lipids with higher T_c. A molecular explanation for this effect may be that bilayers accommodate an increasing number of detergent monomers until saturation occurs, and then lipid–detergent mixed micelles start to form, coexisting with detergent-containing bilayers. An inherent higher degree of molecular disorder may lead to a higher capacity to accommodate detergent molecules while preserving the bilayer structure. Note that all our systems are in the liquid-disordered state, in spite of the various degrees of lipid order probed by DPH polarization. In the presence of cholesterol, among other molecules, PC bilayers exist in the liquid-ordered state, and in that case they are *less* prone to solubilization [12]. Thus, our observations must be taken as valid within the boundaries of the liquid-disordered state, by far the most abundant in biomembranes.

Temperature is known to decrease lipid order in membranes. The effect of temperature on bilayer solubilization by Triton X-100 has not been specifically examined in this work, mainly because it has been the object of other studies [8,12,21]. However, in some cases, solubilization has been studied both at 20 °C and at 45 °C. Results, summed in Table 2, confirm that, at higher temperatures, PC solubilization requires higher amounts of detergent. The relationship between temperature, molecular order, and detergent solubilization has been recently discussed by Lichtenberg et al. [6].

4. Conclusions

The main conclusion of this paper is that, for the solubilization of phosphatidylcholine bilayers in the fluid state (disordered), more detergent (Triton X-100) is required for unsaturated than for saturated phospholipids of the same chain length. For systems within the boundaries of the liquid-disordered state, hydrocarbon chain molecular order, as measured by DPH fluorescence polarization, facilitates solubilization. Phospholipid chain length, in itself, is not a major determinant of the amount of detergent required for solubilization. Other factors, e.g., *cis–trans* isomery, are relevant as far as they influence molecular order in the bilayer. Since all observations have been carried out under equilibrium conditions, the reasons for the different amounts of detergent required for solubilization appear to be thermodynamic in nature, and refer essentially to the different capacities of bilayers of different compositions to accommodate detergent monomers without giving rise to lipid–detergent mixed micelles. In general, less ordered bilayers have a greater capacity for detergent monomers.

Acknowledgments

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