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Abstract

The structural transition stages induced as a result of interaction at 4°C of the Triton X-100 with large unilamellar vesicles were studied by means of a sucrose flotation procedure similar to that used to isolate biological detergent resistant membranes. Flotation of lipid structures after centrifugation was determined on the basis of the [1α, 2α (n)-^{3}H]-Cholesterol content of each fraction of a 40-35-5 % sucrose density gradient. We measured the amount of Triton X-100 insoluble floating fractions (TIFFs) for different lipid compositions of large unilamellar vesicles and different effective detergent to lipid ratios. At 4°C and for two component lipid membrane (PC/SM 2:1 mol/mol), an effective detergent to lipid ratio of 50 is necessary to complete membrane solubilization. When liquid-ordered and liquid-disordered phase domains coexist in the vesicle membrane (PC/SM/Chol 53:27:20 mol/mol), complete solubilization occurs at higher effective detergent to lipid ratio. This is consistent with a higher resistance of the liquid-ordered phase to detergent extraction. Nevertheless, in the case of heterogeneous (l_o/l_d phase) vesicles, and for a range of effective detergent to lipid ratios promoting incomplete solubilization, we detected in TIFF intermediate density structures which did not exist for two component lipid membranes (PC/SM 2:1 mol/mol). We interpreted these results in relation with recent findings of our group and propose a mechanism for heterogeneous large unilamellar vesicle solubilization. We show that for lipid bilayers exhibiting l_o/l_d phase co-existence, a specific effective detergent to lipid ratio allowing the isolation of pre-existing tightly packed ordered domains can be found, but, in any case, certain amount of the detergent is presented in floating fraction membranes.

Key words:

Rafts, DRMs, l_o-phase domains, Triton X-100, LUV

Abbreviations used:

DRMs, detergent resistant membranes; PC, egg yolk L-α-phosphatidylcholine; SM, egg yolk sphingomyelin; Chol, cholesterol; l_d and l_o, liquid-disordered and liquid-ordered (cholesterol containing) L_α phases; TX-100, Triton X-100; LUV, large unilamellar vesicle; TIFFs, Triton X-100 insoluble floating fractions; [^{3}H]Chol, [1α, 2α (n)-^{3}H] Cholesterol

1. Introduction

Detergent interactions with membranes were actively explored during the past decades after it was first appreciated that detergent solubilization was useful for isolating and characterizing membrane proteins [1,2]. More recently, it was shown that detergent can solubilize differentially membrane domains in different physical states [3]. The discovery of these detergent resistant membranes (DRMs) led to a novel description of the cellular membrane emphasizing that the self-organizing properties of particular lipids drive the formation of specialized domains called rafts [4]. This lateral organization may have a huge role in cell signaling since the different lipid environments lead to an inhomogeneous membrane protein distribution [5-7]. Measurement of the insolubility of lipids in detergents has therefore become a useful method for probing the structure of biological membranes. Another important finding was that DRMs are enriched in sphingomyelin (SM) and cholesterol (Chol) [8]. For particular composition and temperature intervals, model membranes present as well lateral phase separation of liquid-disordered phase (l_d) and liquid-ordered phase (l_o) rich in SM and Chol [9-11]. Because of these similarities, rafts are identified with l_o-phase domains [12,13] although the composition of the later, according to phase diagrams made on model membrane could be multiple. In any case, these results stimulated many studies on artificial lipid membranes, exhibiting l_o/l_d phase co-existence as minimal model for raft-like domains containing membrane [10,14-17].

Despite the elements described above, biological membrane rafts still remain quite enigmatic as structural entities so that their existence has been challenged [13,18,19]. As a matter of fact, non destructive direct approaches (fluorescent microscopy or single particle tracking for example) have not yet yielded fully consistent results [20-22]. The detergent approach which identifies rafts as DRMs, i.e membrane fractions which resist to solubilization by detergent (Triton X-100, Brij 98, LUBROL...) lead to a host of potential problems. First of all, performing solubilization at low temperature (4°C) can lead to an overestimation of the amount of raft material. Very low temperature can indeed modify the physical state of the lipids of cellular membrane increasing or, even inducing, lateral lipid phase separation which may not have been present at 37°C [13]. Moreover, lipid and protein compositions and amounts find in DRMs depend strongly on the nature of the detergent used and other experimental conditions [23]. This raises the major question as whether DRMs constitute an accurate measurement of the amount of the raft domains prior to detergent addition. To answer this question, model membrane exhibiting l_o/l_d phase co-existence appears to be useful. The degree of lipid insolubility depends on the stability of lipid-lipid interactions relative to lipid-detergent interactions. Therefore, a minimal system composed exclusively of phosphatidylcholine (PC), SM and Chol can lead to a better understanding of the detergent extraction process which is view in fact, as a selective solubilization of the lipid bilayer [24-28].

The solubilization of homogeneous lipid membranes is usually described by a three-stage model [29]. In the first stage, at low effective detergent to lipid ratios, the detergent is distributed between the aqueous medium (as a monomer) and the lipid bilayers. When a first critical detergent mole fraction is reached, mixed detergent-lipid micelles appeared in coexistence with detergent-saturated membranes. This is a state of partial or incomplete solubilization. The third stage is reached when complete solubilization occurs and all membranes disappear. So, the key parameter controlling the degree of solubilization is the effective detergent to lipid ratio which depends strongly upon the physical state of the lipid bilayer and as a consequence, of the lipid composition and temperature [24,26,30]. The situation becomes more complex – but more 'biologically relevant' – when the bilayer exhibits l_o/l_d phase co-existence because one have to consider the different lipid phase-detergent interactions. l_o membrane domain has been shown to be more resistant to solubilization by detergents than the l_d membrane domain because of a

specific SM/Chol interaction [26]. This finding is consistent with the shared idea that 'DRM = l_o domain'. However, Heerklotz et al. [24,28,31] have shown that Triton X-100 (TX-100) could promote – or even more induce – the l_o membrane domain formation. Nonideal interactions between detergents and order-preferring lipids are a possible driving force for such an effect. On the basis of such conflicting results, one can see that detergent extraction in order to evaluate domain structure and compositions prior to detergent addition did not yet provide actually definitive answers to question about raft existence.

In the present work, the structural transitions induced by the interaction of the TX-100 with large unilamellar vesicles at 4°C were studied by means of a sucrose flotation procedure similar to that used to isolate biological DRMs. Flotation of lipid structures after centrifugation was determined on the basis of the [1α, 2α (n)-^{3}H] Cholesterol ([^{3}H]Chol) content of each fraction (1-10) of a 40-35-5 % sucrose density gradient. We measured the amount of Triton X-100 insoluble floating fractions (TIFFs) at varying effective detergent to lipid ratio and for different physical states of the lipid bilayers. Our results show that depending on the effective detergent to lipid ratio, it is possible to obtain floating fractions and complete solubilization of the lipid bilayers for two component lipid membranes (PC/SM 2:1 mol/mol) and heterogeneous (l_o/l_d phase co-existence) membranes. However, when the bilayer exhibits l_o/l_d phase co-existence, the solubilization process is much more complex. We interpreted our results with regards of recent findings of our group [25]. We tried to evaluate the appropriate effective detergent to lipid ratio which permits us to obtain the TIFF corresponding as much as possible to the l_o domains existing in the LUV membranes before detergent addition.

2. Materials and Methods

2.1. Chemicals

Egg yolk phosphatidylcholine (PC), egg yolk sphingomyelin (SM), Cholesterol (Chol) and Triton X-100 were purchased from Sigma (France). Radiolabeled cholesterol [1α, 2α (n)-^{3}H] Cholesterol was obtained from Amersham Biosciences (Buckinghamshire, UK).

2.2. Preparation of liposomes

Large unilamellar vesicles (LUVs) were made using the extrusion method [32]. Briefly, samples were prepared by mixing the indicated lipid stock solutions to obtain the chosen compositions. Thereafter, lipid mixtures were dried from a chloroform-methanol solution under a stream of nitrogen and further dried under vacuum for at least 1 h. The lipid films were hydrated in a TNC buffer (Tris-HCl 20mM, NaCl 150mM, $CaCl_2$ 1mM, pH 7.5) to yield a lipid concentration of 1 mM. The samples were then vigorously vortexed for 30 seconds at room temperature (23°C), and heated at 65°C for 30 minutes, sonicated, vortexed again for 30 seconds to ensure more uniform vesicles dispersion and placed again to 65°C bath for 15 minutes (exceeding the main phase transition temperature of lipid mixture components as well as that of immiscibility in order to obtain homogeneous lipid mixtures for the subsequent LUV preparation). The multilamellar vesicles were then extruded with a LiposoFast small-volume extruder equipped with polycarbonate filters (Avestin, Ottawa, Canada) with: 12 extrusions through 800 nm followed by 21 extrusions through 100 nm filters. LUV samples were kept at 4°C, protected from light, until use. Radiolabeled LUVs were prepared as described above except

a trace amount (1 μCi/ml) of [^{3}H]Chol (35 Ci/mmol, Amersham Biosciences, Roosendaal, The Netherlands) was added to the lipid mixtures before drying. In the binary and ternary systems made of PC/SM, or PC/SM/Chol, the ratio PC/SM was always 2:1 mol/mol.

2.3. Triton X-100 insoluble floating fraction

TIFF were obtained from LUVs containing [^{3}H]Chol (0.8 ml, 300μM lipids) by treatment with different % of TX-100 (w/v) as indicated, in TNC (Tris-HCl 20mM, NaCl 150mM, $CaCl_2$ 1mM) buffer pH 7.5, at 4°C for 30 min or otherwise stated. Subsequently, the detergent-LUV sample was mixed with 80% sucrose (w/v) to a final concentration of 40% sucrose in a final volume of 1.6 ml and placed on the bottom of an ultracentrifuge tube. Then, an 1.6 ml layer of 35 % sucrose (w/v) in TNC, and an 1.6 ml layer of 5 % sucrose (w/v) in TNC were over layered in order to make the density gradient. The gradients were then subjected to ultracentrifugation in a Beckman SW55 Ti rotor for 18h at 180 000 × *g* at 4°C. After centrifugation, fractions of 500 μl were collected, starting from the top of the gradient. Aliquots were removed to determine the amount of [^{3}H]Chol in each fraction by liquid scintillation counting. Experiments were reproduced at least twice. Results are quite reproducible if the different fractions after ultracentrifugation are carefully collected. For 0.75 % TX-100 (w/v), no substantial variation of the [^{3}H]Chol extent was detected when LUVs were treated during 5, 15, 30 or 120 min (data not shown). To evaluate the density in each collected fraction, the refractive index and % sucrose by weight were read using a refractometer (Atago, Japan). The corresponding densities were then determined using a sucrose density index.

3. Results

3.1. Evaluation of the presence of TIFFs in LUVs

To evaluate the presence of Triton X-100 resistant membranes in liposomes, LUVs (300 μM lipids) were treated with TX-100 at 4°C for 30 min and subjected to a density gradient flotation analysis. Triton X-100 insoluble fractions floating to the top of a 40-35-5% sucrose density gradient were detected by the [^{3}H]Chol initially incorporated in the LUVs.

When no Triton was added to the vesicles (Fig. 1), almost 90 % of the [^{3}H]Chol total content was detected in the first four fractions (1-4). These vesicle containing fractions correspond to the lowest density fractions of the sucrose gradient (Fig. 2). It is probably due to the very low membrane permeability for sucrose in the absence of detergent. So, buffer containing vesicles are simply floating in the sucrose solution. The ten percents remaining distributed into the fractions 5 to 10 may come from micelles or “high density structures” existing after the extrusion process which remained in the bottom of the ultracentrifugation tube. Consequently, the [^{3}H]Chol content calculated in the fraction 5 to 10 when no Triton was added, was considered as “background noise” and systematically substracted from the total [^{3}H]Chol content. When Triton was added to the vesicles – in non complete solubilizing amount –, the total [^{3}H]Chol content could be separated in two major contribution (Fig.1, for data ▲ and ○). The first one, corresponding to the fractions 1 to 4 i.e. to the densities 1.029 to 1.076 kg/m^3 (Fig. 2), was considered as TIFFs. The second one (fractions 5 to 10, densities 1.105 to 1.160 kg/m^3, Fig. 2) correspond to the detergent soluble fractions of the membrane and is mainly composed of mixed detergent-lipid micelles. One example is given in Fig. 1 for 0.8 % Triton X-100 (w/v) and two different sorts of liposomes. One can see that in comparison with the case without Triton added,

the [^{3}H]Chol content increased in the fractions (5-10) and decreased in the fractions (1-4). This is a signature of a partial solubilization of the vesicle membranes [33]. Furthermore, the total [^{3}H]Chol content measured in TIFFs of "pro-rafts" vesicles (PC/SM/Chol 53:27:20 mol/mol) – i.e. bilayer exhibiting l_o/l_d phase co-existence [11] – was higher than the one with two component lipid vesicles (PC/SM 2:1 mol/mol). The total membrane solubilization occurred when no, or very few, radiolabeled Chol was detected in the four first fractions (example in Fig. 1, data ◊).

3.2. TX-100 concentration dependence of TIFF

To investigate in more details the resistance of membranes to detergent solubilization we used the same flotation procedure, but with variable amounts of Triton X-100. Experiments were done with two component lipid vesicles and for heterogeneous, domain-forming membranes which solubilization followed a more complicated way [28].

3.2.1. Two component lipid membranes

For the PC/SM 2:1 mol/mol vesicles, we studied the Triton X-100 effect for the range 0 to 0.8 % (w/v). The lipid amount was fixed at 300 µM. Fig. 3A presents the % [^{3}H]Chol content of total for each fraction of the sucrose density gradient for different TX-100 percentages. In comparison with the case without Triton added (Fig. 3A, data ♦), it can be remarked that the initial floating structures are partly solubilized and that the TIFFs are now principally composed of structures (Fig.3A, data □, ▲, ○ and ●) of fraction (2) density. The partial solubilization of the initial floating structures indicate that, till 0.2 % TX-100 (w/v), we are above the effective saturating detergent to lipid ratio. So, these structures should correspond to smallest vesicles than the initial ones which membranes are saturated with detergent. Fig. 3B summarizes the results obtained (% TIFF content of total) for the different Triton percentages used. Increasing the TX-100 amount lead little by little to the disappearance of TIFFs. For the rest, the decrease is nearly linear (Fig. 6B, data ○). For 0.8 % (w/v) TX-100, the amount of floating fractions is weak that point to the total solubilization of the membrane may occur around 0.9 %.

In the case of pure l_0 phase vesicles (SM/Chol 1:1 mol/mol), no solubilization occurred up to 2.5 % TX-100 w/v (more than 90% of the [^{3}H] total content was found in the Triton insoluble floating fractions, data not shown).

3.2.2. Heterogeneous (l_o/l_d phase) LUVs

We used PC/SM/Chol 53:27:20 mol/mol vesicles whose membranes exhibit a l_o/l_d phase co-existence [11,17,25]. The Triton X-100 range was 0 to 1.3 % (w/v) for a fixed amount of lipid of 300µM. Fig. 4A presents the results obtained with the different TX-100 percentages used. As for the two component lipid vesicles (PC/SM 2:1 mol/mol) (Fig. 3B), one can see that the TIFF amount (fractions 1 to 4) decreases when the effective detergent to lipid ratio increases (Fig. 4A, grey bars). The lipid membrane is first partially solubilized – indicating that at least the effective saturating detergent to lipid ratio of the l_d-phase is reached – and then totally micellized at 1.2 % TX-100 (w/v). The total solubilization of the membranes point to that at 1.2 % TX-100 (w/v), the effective saturating detergent to lipid ratio of the l_o-phase is also reached. So, the solubilization process of the heterogeneous lipid bilayer seems to be similar to the two component lipid one but with a shift to higher percentage of TX-100. This is consistent with the higher detergent resistance of the l_0-phase usually described in the literature [26].

But, there are also important differences between the solubilization processes if a precise analysis of the floating fractions is done. Addition of small amounts of TX-100 till 0.25 % (w/v) shifts the initial lipid structures to higher density ones (around 1.076 kg/m^3, fraction 4) (Fig.4B).

For 0.1 to 0.25 % TX-100 (w/v), the [^{3}H]Chol of floating fractions is principally found in the fourth fraction corresponding to medium density structures (1.076 kg/m^3, Fig. 2) that did not exist for two component lipid vesicles (PC/SM 2:1 mol/mol). When the TX-100 amount still increases (0.4 to 0.6 % TX-100 w/v), there is a slight translation of this maximum toward the fraction (2) i.e. the low density structures. This phenomenon is probably due to an increasing incorporation of the detergent in the l_o domain membranes indicating that the effective detergent to lipid ratio is still below the l_o-phase saturating one. The results described above are brought together in the Fig. 4A (white bars) where one can see that, for detergent amount in the considered range (0.05 to 0.6 % w/v), the [^{3}H]Chol content of the fractions (1-3) stay more or less constant whereas there is a progressive decrease of the [^{3}H]Chol content of the fraction four (differences between grey and white bars) corresponding to the medium density structure signal. Above 0.6 % TX-100 (w/v), the [^{3}H]Chol content of the fractions (1-3) and (1-4) are almost identical and decrease till their annulments (Fig. 4A). It can be remarked that this decrease is done without further density shift of the floating fraction structures (Fig. 4C) indicating that a progressive solubilization of the existing floating structures at 0.6 %TX-100 (w/v) takes place. The effective saturating detergent to lipid ratio of the l_o phase is now reached.

The totality of the results are summarized in the Fig. 5. This figure permits us to emphasize that is possible to find a particular detergent concentration (around 0.6 % TX-100 w/v for 300µM lipid and detergent extraction at 4°C) for which only l_o-domains of PC/SM/Chol 53:27:20 mol/mol vesicles are found as TIFFs. However, these domains, almost saturated with TX-100, are obviously not exactly the same than those existing before the detergent treatment.

4. Discussion

The isolation of resistant membrane fraction from a membrane depends on a number of parameters including the nature of the detergent, the temperature, the physical state of the lipid bilayer [23,25]…On the other hand, for a specific detergent, all these parameters can be reduce in one key variable controlling the degree of solubilization: the effective detergent to lipid ratio since the latter depends strongly upon the physical state of the lipid bilayer and as a consequence, of the lipid composition and temperature [24,26,30].

Our results show that depending on the effective detergent to lipid ratio, it is possible to obtain partial and complete solubilization of the lipid membranes. Floating fractions resulting from a detergent treatment could be obtained whatever the physical state of the membrane studied here (Fig. 3B and 4A). Nevertheless, our goal is to find floating fractions originating from pre-existing membrane structures i.e. the l_o domains of the LUVs. So, the key point is to find – if possible – the appropriate effective detergent to lipid ratio permitting this. The existence of such a ratio is under debate because recent results show that TX-100 could promote – or even more induce – the l_o membrane domain formation [22,28.31]. This idea is based on the assumption that ordered and disordered domains in membranes are in equilibrium and that consequently, the modification of the l_d-domains during TX-100 incorporation will also necessarily alter the l_o-domains. However, recent results of our group [25] showed that addition of TX-100 to giant vesicles exhibiting an l_o/l_d phase co-existence induced the l_o-domains to bud and form separate vesicles. Whatever is the structure of disconnected objects for LUVs (vesicles or bicelles), the physical disconnection of the two phases forbid any important l_o-domain composition alteration and thus, allows the existence of such a ratio.

The first idea is that two mechanisms take place simultaneously in the solubilization process of domain-forming LUVs. Below 0.6 % TX-100 (w/v), the l_d-phase membrane fraction is

solubilized little by little (we are above the effective saturating detergent to lipid ratio of the l_d phase) while the l_o-phase membrane fraction is loaded by TX-100 (we are still below the effective saturating detergent to lipid ratio of the l_o-phase). This is consistent with the increase of the [^{3}H]Chol content of the non-floating fractions (5-10) (Fig.4B) and with the density shift toward the low densities of the floating fraction structures (1-4) (Fig.4B). Around 0.6 % TX-100 (w/v), the l_d-phase is now totally solubilized and the effective saturating detergent to lipid ratio of the l_o-phase is reached (no further density shift of the floating structures, Fig. 4C). Then, above 0.6 % TX-100 (w/v), the decrease of the [^{3}H]Chol content of the floating fractions (Fig. 4C) indicates a progressive solubilization of the existing floating structures. Consequently, we consider that a detergent to lipid ratio around 0.6 % TX-100 (w/v) is appropriate to obtain floating fractions coming from pre-existing membrane structures i.e. TX-100 loaded l_o-domains. Such an explanation is borne out by the representations in Fig. 6A and 6B. In the Fig. 6A, the % TIFF of total in the fractions (1-3) is plotted according to a particular effective detergent to lipid ratio R_e^*. It should be noted here that the amount of lipid present in the l_o-phase is not consider in order to calculate this particular ratio R_e^*. So, for LUVs PC/SM 2:1 mol/mol, all the lipids are taken into account. For heterogeneous LUVs, because the surface fraction covered by the l_o-domains is about 20 % of the total (studies involving the fluorescence self-quenching of C_{12}NBD-PC, [34]), only 80 % of the amount of lipid is considering to calculate this particular effective detergent to lipid ratio (lipids present in the l_d-phase). Comparatively to the figure 5 (data ■), this correspond to a shift of the dotted curve for heterogeneous LUVs (Fig. 6A, data ■) toward higher effective detergent to lipid ratio. Consequently, one can see that the needed detergent content that completely solubilize the LUVs PC/SM 2:1 mol/mol correspond more or less to the detergent content needed to begin the solubilization of the floating fractions (1-3) of domain-forming LUVs. This transition occurs around 0.6 % TX-100 w/v and this is consistent with our conclusions. Furthermore, one can notice in Fig. 6B that the maximum [^{3}H]Chol content of the fraction (2) is also reached for 0.6 % TX-100 (w/v). Further experiments will allow us to determine more precisely this maximum. We expect that the effective detergent to lipid ratio corresponding to the maximum of such a curve may be the appropriate ratio which allows to obtain the TIFF corresponding as much as possible to the l_o domains existing in the LUV membranes before detergent addition.

We have presented here an ideal scenario which allows us to determine the appropriate effective detergent to lipid ratio which permits the isolation of the l_0 domains of an heterogeneous vesicle. This scenario does not take into account the different and competitive kinetic factors between physical disconnection and possible phase re-equilibration of the l_o phase domains under the TX-100 interactions.

5. Conclusion

In this paper, we studied the behaviors of lipid membranes upon detergent solubilization. We showed that depending to the TX-100 to lipid ratio, lipid membranes could be partially or totally solubilized. Nevertheless, for lipid bilayers exhibiting l_o/l_d phase co-existence, a particular ratio allowing the isolation of the tightly packed ordered domains existing before the detergent treatment could be found, but, in any case, certain amount of the detergent is presented in floating fraction membranes.

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Figure Captions:

Fig. 1. Flotation of Triton insoluble fractions after treatment of LUVs (300 µM lipid) at 4°C with different Triton X-100 percentages (w/v). 0%, LUVs PC/SM 2:1 mol/mol (□). 0.8%, LUVs PC/SM 2:1 mol/mol (○) and LUVs PC/SM/Chol 53:27:20 mol/mol (▲). 1.3 %, LUVs PC/SM/Chol 53:27:20 mol/mol (◊). Flotation of lipid structures after centrifugation was determined on the basis of the [^{3}H]Chol content of each fraction (1-10). The density corresponding of each fraction is given in Fig.2.

Fig. 2. % sucrose by weight (●) and density (□) of each gradient fraction after centrifugation. The refractive index and % sucrose were read using a refractometer. The corresponding densities were then determined using a sucrose density index. Fractions (1-3), (4) and (1-4) are called respectively *Low density*, *Medium density* and *Low + medium density* fractions.

Fig. 3. Flotation of TIFFs after treatment of LUVs PC/SM 2:1 mol/mol (300 µM lipid) with Triton X-100 at 4°C. Flotation of lipid structures after centrifugation was determined on the basis of the [^{3}H]Chol content of each fraction (1-10).
(A) % [^{3}H]Chol of total for each fraction: (♦) 0 % TX-100 (w/v). (□) 0.2 % TX-100 (w/v). (▲) 0.4 % Triton X-100 (w/v). (○) 0.6 % TX-100 (w/v). (●) 0.8 % TX-100 (w/v).
(**B**) % TIFF of total in the fractions (1-4) (Low density + medium density, grey bars) of the sucrose density gradient after treatment of LUVs (PC/SM 2:1 mol/mol) with Triton X-100 at 4°C. Error bars represent the standard deviation between two experiments.

Fig. 4. Flotation of TIFFs after treatment of LUVs PC/SM/Chol 53:27:20 mol/mol (300µM lipid) with Triton X-100 at 4°C. Flotation of lipid structures after centrifugation was determined on the basis of the [^{3}H]Chol content of each fraction (1-10).
(**A**) % TIFF of total in the fractions 1 to 4 (Low + Medium density fractions, grey bars) and 1 to 3 (Low density fractions, white bars) of the sucrose density gradient. Error bars represent the standard deviation between two experiments.
(**B**) % [^{3}H]Chol of total for each fraction: (♦) 0.1 % TX-100 (w/v). (□) 0.17 % TX-100 (w/v). (▲) 0.25 % Triton X-100 (w/v). (○) 0.4 % TX-100 (w/v). (■) 0.5 % TX-100 (w/v). (●) 0.6 % TX-100 (w/v).
(**C**) % [^{3}H]Chol of total for each fraction: (♦) 0.6 % TX-100 (w/v). (□) 0.7 % TX-100 (w/v). (▲) 0.8 % Triton X-100 (w/v). (○) 0.9 % TX-100 (w/v). (●) 1.0 % TX-100 (w/v).

Fig. 5. % TIFF of total in the fractions 1 to 4 (◊), 1 to 3 (■) and 4 (○) of the sucrose density gradient after treatment of LUVs PC/SM/Chol 53:27:20 mol/mol with Triton X-100 at 4°C. Error bars represent the standard deviation between two experiments.

Fig. 6. (**A**) % TIFF of total in fractions (1-3) versus a particular effective detergent to lipid ratio R_e^*. R_e^* is calculate considering only the lipids that are not in the l_o phase (100 % for LUVs PC/SM 2:1 mol/mol and 80 % for LUVs PC/SM/Chol 53:27:20 mol/mol). (■) LUVs PC/SM/Chol (53:27:20 mol/mol). (○) LUVs PC/SM (2:1) mol/mol.
(**B**) % [^{3}H]Chol content of fraction (2) versus % TX-100 (w/v) for LUVs PC/SM/Chol 53:27:20 mol/mol.