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Two distinct mechanisms of vesicle-to-micelle and micelle-to-vesicle transition are mediated by the packing parameter of phospholipid–detergent systems

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Abstract

The detergent solubilization and reformation of phospholipid vesicles was studied for various detergents. Two distinct mechanisms of vesicle-to-micelle and micelle-to-vesicle transition were observed by turbidimetry and cryo-electron microscopy. The first mechanism involves fast solubilization of phospholipids and occurs via open vesicular intermediates. The reverse process, micelle-to-vesicle transition, mimics the vesicle-to-micelle transition. In the second mechanism the solubilization is a slow process that proceeds via micelles that pinch off from closed vesicles. During vesicle reformation, the micelle-to-vesicle transition, a large number of densely packed multilamellar vesicles are formed. The route used, for solubilization and reformation, by a given detergent–phospholipid combination is critically dependent on the overall packing parameter of the detergent-saturated phospholipid membranes. By a change of the overall packing parameter the solubilization and or reformation mechanism could be changed. All five detergents tested fit within the proposed model. With two detergents the mechanism could be changed by changing the phospholipid composition or the medium conditions.

Keywords: Phospholipid; Detergent; Solubilization; Micelle; Vesicle; Cryo-electron microscopy

1. Introduction

Phospholipids, in liposomes and as planer bilayers, are nowadays widely used to study membrane-related biological processes. Very often detergents are used to prepare the liposomes from phospholipids or to reconstitute membrane proteins into phospholipid bilayers for functional or structural studies. Although the solubilization of phospholipids by detergents has been studied extensively, the reverse process, the reformation of bilayers upon detergent depletion is underdeveloped. Moreover, the non-equilibrium behaviour of the micelle-to-vesicle transition is not well understood with respect to the reformation of bilayers although the micelle-to-vesicle transition has long been recognized as the state of importance for the reconstitution of membrane proteins [1]. The solubilization of phospholipids by detergents has been subjected to many studies, and the different stages during the solubilization are often characterized on the basis of turbidity measurements [2–8]. In general, three stages are recognized during the detergent-induced solubilization of phospholipids [2,5]. In stage I detergent molecules insert into the phospholipid bilayer until the bilayer is saturated (R_{sat}). R_{sat} is generally reflected by a maximum turbidity due to swelling of the membrane or by fusion of the liposomes. Upon a further increase of the detergent concentration the bilayers will start to disintegrate and bilayers coexist with detergent phospholipid mixed micelles (stage II). The detergent concentration at which the solubilization is complete (R_{sol}) separates stage II and stage III where only detergent–phospholipid mixed micelles exist.

The intermediate state between bilayer and mixed-micelles, between R_{sat} and R_{sol} [9], is crucial for understanding the mechanism of vesicle solubilization. Previously, specific differences were found in the solubilization time and based on these findings two different mechanisms of vesicle solubilization were described [10]. One reaction assumes fast solubilization via open intermediates with detergent molecules acting from both sides of

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the phospholipid bilayer, called transbilayer solubilization. The other slow reaction goes via closed vesicles from which detergent phospholipid micelles escape from the outer monolayer of the vesicle, called micellar solubilization.

Here the differences between the two mechanisms are studied extensively by turbidimetry and cryo-electron microscopy and extended from solubilization to the reverse process of vesicle reformation. Although turbidimetry is rather non-specific, in combination with cryo-electron microscopy it gives a valuable indication in the solubilization progress. Whereas many studies use phospholipid extracts from plant or bacteria, which are mixtures of different acyl-chains, here pure synthetic phospholipids with unsaturated acyl-chains were used, providing phospholipid vesicles in the fluid (L_α) phase. The detergents that were used are those which have been used frequently for the purification and reconstitution of membrane proteins.

It cannot be guaranteed that all experiments have been performed under equilibrium conditions. However, our experimental conditions are those used in practice.

2. Materials and methods

2.1. Materials

n-Decylmaltoside (DM), n-dodecylmaltoside (DDM) 3-[(3-cholamidopropyl)-dimethylammonio]-propanesulfonate (CHAPS), octyl-β-d-glucopyranoside (βOG), octyl-β-d-thioglucopyranoside (OTG) and Triton X-100 were obtained from Sigma. Dioleoyl-phosphatidyl-choline (DOPC) and dioleoyl-phosphatidyl-ethanolamine (DOPE) were purchased from Avanti Polar Lipids (Alabaster, AL). Bio-beads SM2 (Bio-Rad, Veenendaal, The Netherlands) were washed with methanol and rinsed with double-distilled water prior to use. Nile Red was obtained from ACROS (Landsmeer, The Netherlands). All other chemicals were of the highest purity.

2.2. Vesicle-to-micelle transition upon detergent increase

Aliquots of phospholipids were mixed from chloroform stock solutions and dried under nitrogen and subsequently placed under vacuum to remove traces of chloroform. Then the phospholipids were hydrated in a buffer containing 10 mM HEPES, 100 mM NaCl, pH 7, to a final concentration of 2.5 mM and freeze–thawed twice. Unilamellar liposomes were prepared by extruding the suspension 11 times through 200 nm polycarbonate filters. Detergent was added in a stepwise manner from a 100-mg/ml stock solution in H_2O. Time was allowed between successive additions to reach a stable turbidity level. It must be mentioned that in some cases solubilization is slow and we are therefore working in non-equilibrium conditions. At different stages in the solubilization samples have been equilibrated for days, up to weeks unless no difference was observed. The solubilization of liposomes was monitored by measuring the turbidity (OD at 540 nm) continuously on a UV/VIS spectrophotometer (Ultrospec 3000, Pharmacia Biotech). In some cases intermediate stages are equilibrated up to several weeks and observed in time.

2.3. Micelle-to-vesicle transition upon detergent removal

After complete solubilization of phospholipids was reached, a carefully weighted amount of bio-beads was added to remove the detergent. In general the capacity is 0.04 g detergent per gram of wet bio-beads. The bio-beads were added either all at once or in small portions, dependent on the detergent removal rate and the course of events. Normally vesicle reformation took 1 h. During the micelle to vesicle transition samples have been equilibrated for weeks to check for non-equilibrium results. Detergent removal was done under continuous stirring unless otherwise mentioned. At regular time-points the turbidity of the suspension was measured and samples were taken for detergent concentration measurement.

2.4. Measurement of detergent concentration

The detergent concentration was measured by quantitative thin layer chromatography (TLC) according to standard methods [11] as previously described [12]. In brief, samples containing 1–10 μg detergent were deposited on non-activated TLC plates (silica gel 60, aluminium sheets, Merck, Darmstadt, Germany) and developed in chloroform/methanol/water (45/45/10, v/v). On each TLC plate, spots containing a known amount of detergent were deposited for calibration. After development the plates were air dried and stained by spraying with 10% H_2SO_4 in 50% ethanol followed by heating for 15 min at 100 °C. The plates were scanned on a flatbed scanner (Linotype, Saphir Ultra) and the spot intensities were measured using the Scion-image software package (Scion corporation, Frederick, Maryland, USA) [13]. The concentration of Triton X-100 was measured by its UV absorbance (278 nm). Samples containing 0.1–10 mg/ml Triton X-100 were diluted 20–50 times in 2 mg/ml decylmaltoside prior to the measurement, to avoid interference of light scattering from liposomes.

2.5. Cryo-electron microscopy

1–2 μl of a phospholipid–detergent suspension was placed on a glow discharged holey carbon film. The excess of liquid was blotted with a filter paper prior to vitrification in liquid ethane [14,15]. The grids were examined in a Philips CM10, CM 120 or CM 200 (Philips, Eindhoven, The Netherlands) cryo-electron microscope equipped with a Gatan cryo-stage (model 626, Gatan, Pleasanton, CA). Images were recorded on a slow-scan CCD camera under low-dose conditions (model 794 MSC, Gatan, Pleasanton, CA).

2.6. Nile Red fluorescence spectroscopy

Nile Red was found to be very useful to determine the vesicle-to-micelle transition [16,17]. A 2.5-mM stock solution of Nile Red in ethanol was added to a 0.25-mM DOPC vesicle solution at a ratio of 1:2000. Nile Red fluorescence was measured on a SPF-500c spectrofluorimeter (SLM Aminco) at 25 °C, using an excitation wavelength of 590 nm. The maximum emission wavelength (λ_{max}) of Nile Red was determined by log-normal fit of the emission spectrum. Although light scattering does not influence the maximum emission of Nile Red [16], the phospholipid concentration was kept as low as possible (0.25 mM).

3. Results

3.1. Vesicle-to-micelle transition

Upon stepwise solubilization of liposomes by different detergents two aspects attract the attention. The turbidity around R_{sat} and the time that is needed to reach a stable turbidity level after detergent addition between R_{sat} and R_{sol}. The turbidity around R_{sat} is either around the same level as the starting solution or is extremely high. The detergents that give only a slight increase or decrease of the turbidity around R_{sat} give an almost instantaneous turbidity drop upon an increase of the detergent concentration above R_{sat}. The detergents that result in an extremely high turbidity around R_{sat} need a long time (up to 1 h) to reach a stable turbidity level upon a further increase of the detergent concentration. In Fig. 1 an example is given for the two different ways of solubilizing DOPC. In Fig. 1a the turbidity level is displayed as a function of the detergent concentration for Triton X-100 and decylmaltoside. Upon solubilization of DOPC vesicles with Triton X-100 the turbidity around R_{sat} (at 1.6 mM Triton) is almost the same as the starting value. Upon a further increase of the detergent concentration the turbidity drops instantaneously with each addition of the detergent (arrows inset Fig. 1a). Upon solubilization of the

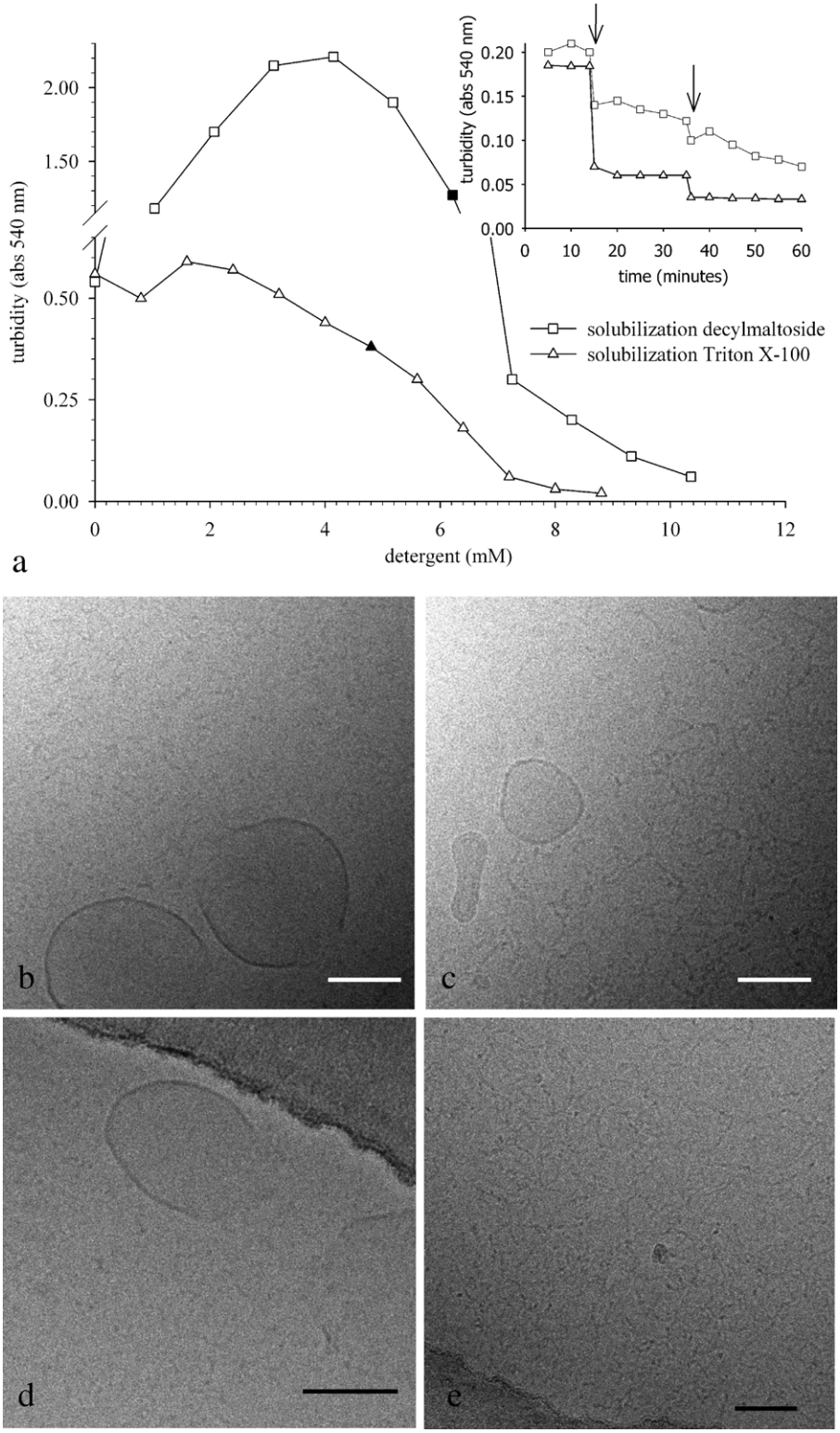

Fig. 1. Solubilization of DOPC (2.5 mM) liposomes by Triton X-100 and decylmaltoside as function of the detergent concentration (a). The turbidity change after increase of the Triton X-100 concentration (0.8 mM/step) is almost instantaneous, whereas the turbidity change after decylmaltoside addition (1 mM/step) (arrows) is slow between R_{sat} and R_{sol} (inset a). At 4.8 and 6.2 mM detergent (filled symbols) samples were taken for cryo-electron microscopy (b–e). Solubilization with Triton X-100, open bilayer fragments and spherical mixed micelles coexists (b), solubilization with decylmaltoside, closed vesicles and worm-like micelles coexists (c). After 1 week equilibration the Triton X-100 solubilized sample did not change. (d) In the decylmaltoside sample the vesicles disappeared and only worm-like micelles remained. Bar 100 nm.

same DOPC vesicles with decylmaltoside the turbidity of the suspension increases tremendously until R_{sat} is reached (around 4 mM). Upon a further increase of the decylmaltoside concentration the turbidity declines slowly in time (inset Fig. 1a). This slow decrease in turbidity is almost independent of the detergent concentration. Cryo-electron microscopy at concentrations between R_{sat} and R_{sol} revealed the difference between the two detergents. In Fig. 1b the intermediate state of DOPC solubilization with Triton X-100 is shown. Open vesicles are seen along with detergent–phospholipid mixed micelles. The intermediate state in the solubilization with decylmaltoside is quite different. Here closed vesicles are seen simultaneously with worm-like micelles (Fig. 1c). After prolonged stabilization of the intermediate state the Triton X-100 solubilised vesicles did not change and open bilayer intermediates are still present (Fig. 1d), whereas in the decylmaltoside solubilised sample only worm-like micelles were found and the closed vesicles have now been completely solubilised into worm-like micelles (Fig. 1e).

For both solubilizations, with Triton X-100 and decylmaltoside a clear vesicle-to-micelle transition was found with the fluorescent probe Nile Red (Fig. 2a). The maximum emission wavelength (λ_{max}) changed from 636 nm for a DOPC bilayer to 641–642 nm for DOPC detergent mixed micelles. The difference between Triton and decylmaltoside is found in stage I of the solubilization. The λ_{max} for Triton X-100 solubilization stays constant until R_{sat} is reached (at 0.48 mM), whereas the λ_{max} for decylmaltoside solubilization decreases before R_{sat} is reached (at 1.7 mM). This decrease is indicative for the exposure of more hydrophobic surface [16] leading to vesicle aggregation (Fig. 2b) and finally vesicle collapse into large multi-layered structures (Fig. 2c), even after prolonged stabilization. This explains the high turbidity increase in stage I upon decylmaltoside solubilization (Fig. 1a). Besides vesicle aggregation the shape of the vesicles changes and protrusions are observed, indicative for an unbalance between lipid and detergent in the inner and outer bilayer leaflet (Fig. 2b) [18].

A different solubilization mechanism for these two detergents is suspected and therefore a series of experiments were undertaken to reveal the differences and the exact mechanism. The size of the starting vesicles was found not to influence the course of events. Small unilamellar vesicles obtained by sonication gave identical results.

3.2. Micelle-to-vesicle transition

The striking difference between the two detergents during solubilization is even more apparent during the reverse process of vesicle reformation upon detergent depletion. During the removal of Triton X-100 with bio-beads the turbidity develops more or less similar to the solubilization, with a small peak at R_{sat} (Fig. 3a). Open bilayers were found during the reformation process any ware between R_{sol} and R_{sat} even after prolonged waiting times (Fig. 3b). The formation of phospholipid liposomes is in general not spontaneous [19] but apparently the continuous stirring is sufficient to make vesicles. The turbidity of the sample that was solubilized by decylmaltoside increased very rapidly when the detergent concentration was lowered to or below R_{sol}. At this point worm-like micelles were found with a high tendency to coalesce (Fig. 3c). Strikingly, the turbidity stayed high during further lowering the detergent concentration even below R_{sat}. Cryo-electron microscopy revealed that after Triton removal unilamellar vesicles of DOPC were formed (Fig. 3d), whereas after decylmaltoside removal densely packed multilamellar vesicles were formed concurrent with small unilamellar or multilamellar vesicles (Fig. 3e).

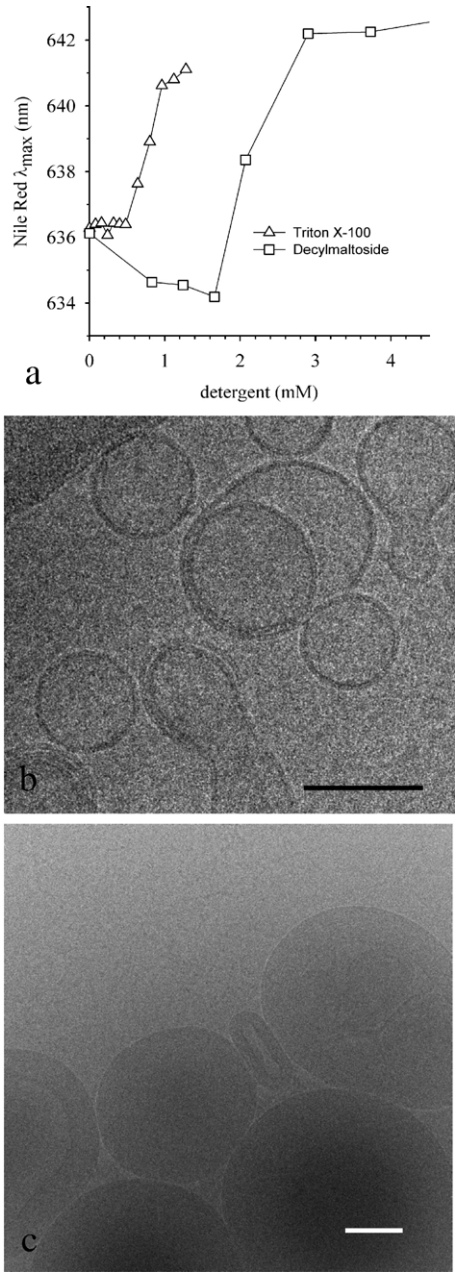

Fig. 2. (a) Nile Red fluorescence (λ_{max}) on 0.25 mM DOPC liposomes. (b) Aggregated liposomes with a changed shape at decylmaltoside concentrations below R_{sat} (2.5 mM DOPC, 2.1 mM decylmaltoside). (c) 2.5 mM DOPC, 3.1 mM decylamtoside (around R_{sat}) stabilized for 4 days. Bar 100 nm.

3.3. Detergent classes

In some cases, small variations in the solubilization or reformation conditions lead to a shift from one mechanism to the other. The difference between βOG and OTG is only the oxygen versus sulphur at the linkage between the hydrophilic sugar headgroup and the hydrophobic tail. This apparently small change gave a complete other behaviour for both vesicle-to-micelle and micelle-to-vesicle transitions (Fig. 4a). A large increase in turbidity around R_{sat} was found upon the solubi-

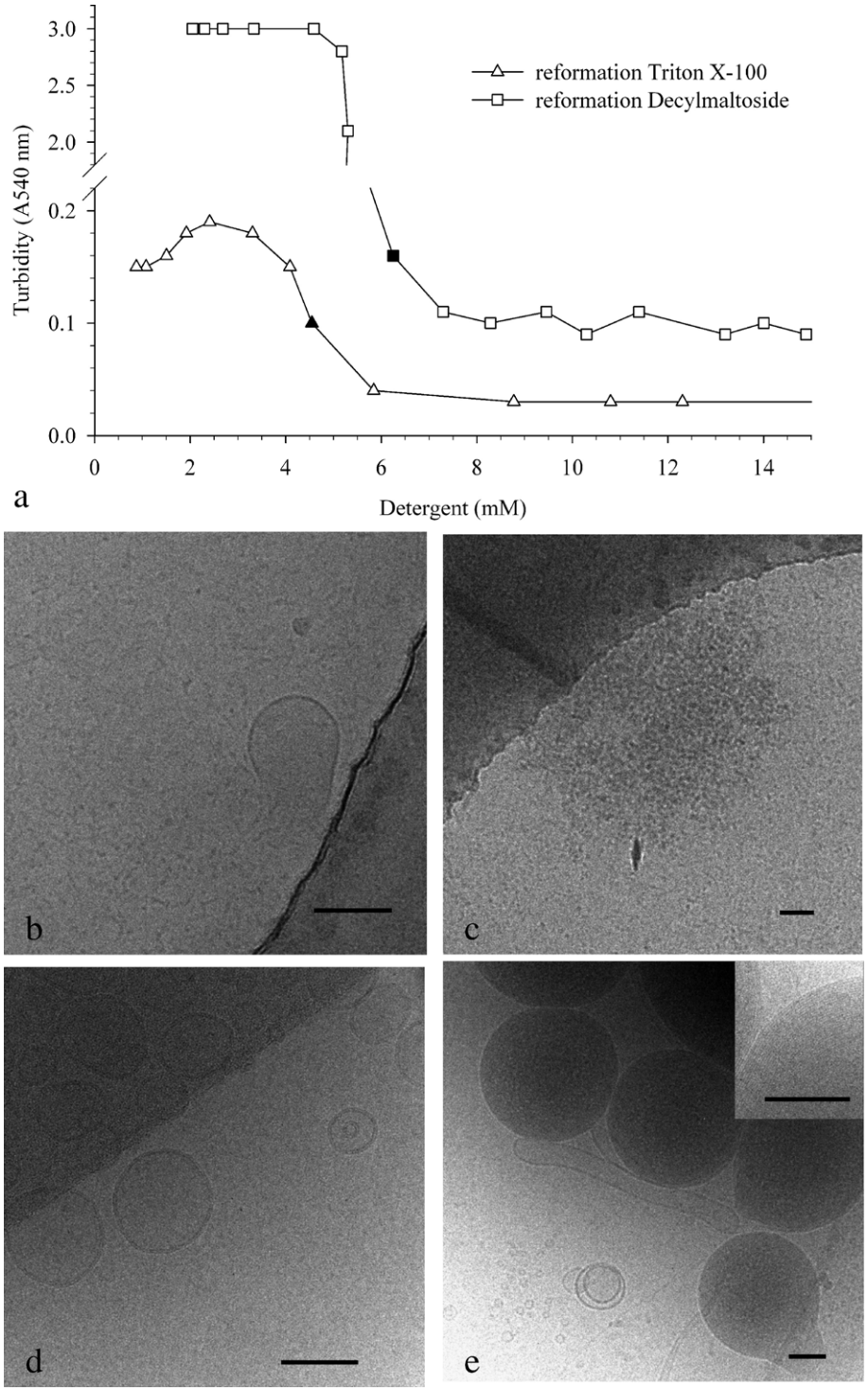

Fig. 3. Reformation of DOPC (2.5 mM) vesicles out off detergent phospholipid mixed micelles. (a) Course of the turbidity of Triton X-100 and decylmaltoside solubilized DOPC. (b) Open vesicle intermediates during the micelle-to-vesicle transition of Triton X-100. (c) Wormlike-like micelles with a strong tendency to coalesce during the micelle-to-vesicle transition of decylmaltoside. (d) Unilamellar liposomes were found after complete Triton X-100 removal. (e) Densely packed multilamellar objects and small unilamellar vesicles were found after decylmaltoside removal. The densely packed multilamellar objects are different from multilamellar vesicles with respect to the packing (inset c). Bar 100 nm.

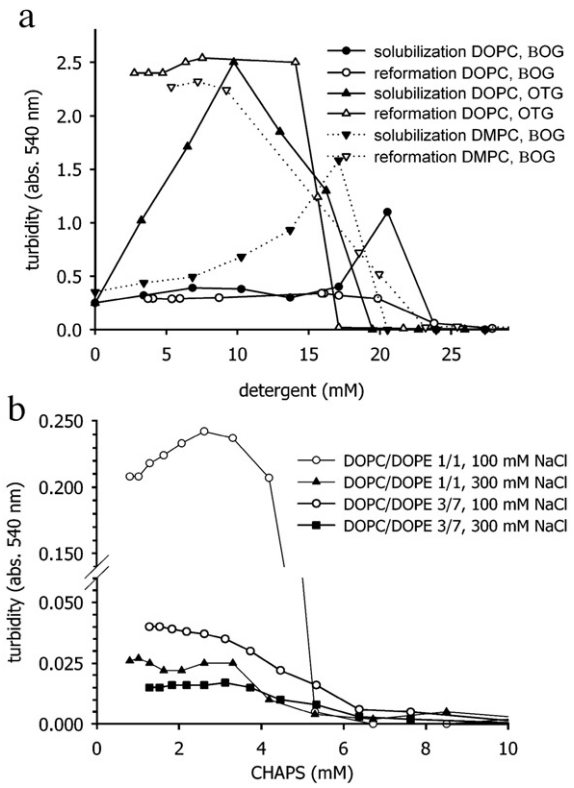

Fig. 4. (a) Solubilization and reformation of DOPC and DMPC (2.5 mM) by octyl-β-D-glucopyranoside (βOG) and ocyl-β-D-thioglucopyranoside (OTG). (b) Solubilization and reformation of DOPC/DOPE (1/1 and 3/7, 2.5 mM) at high (300 mM) and low (100 mM) salt by the detergent CHAPS.

lization with OTG concurrent with the formation of worm-like micelles in the vesicle micelle coexistence region. On the other hand, the solubilization by βOG gave a much smaller turbidity increase and open vesicle intermediates were found coexisting with mixed micelles in the concentration range between R_{sat} and R_{sol} (supporting information). Accordingly unilamellar vesicles were formed after βOG removal, whereas densely packed lamellar droplets were found after OTG removal as reflected by the high turbidity. The solubilization mechanism of βOG changed completely when DOPC was replaced by DMPC. This was seen not only in the speed of solubilization (not shown) but was most pronounced during the micelle-to-vesicle transition upon detergent removal (Fig. 4a). After complete removal of the βOG DOPC reformed into unilamellar vesicles whereas DMPC reformed into densely packed multilamellar liposomes as indicated by the high turbidity.

A similar change between the two different mechanisms was observed during the solubilization of DOPC/DOPE (1/1) by the zwitterionic detergent CHAPS. In this case, increasing the NaCl concentration from 100 to 300 mM could induce the change between the two mechanisms (Fig. 4b). Cryo-electron microscopy of the formed structures after detergent removal showed great resemblance with the structures that were formed after either Triton X-100 or decylmaltoside removal as described before (see supporting information). Besides small changes in the detergent molecule or in the solubilization conditions, changes in the phospholipid composition could bring about the same change from one solubilization mechanism to the other. A similar effect of increasing the salt concentration, as described above, could be induced at low salt concentrations by an increase of the amount of DOPE (Fig. 4b).

4. Discussion

On the basis of turbidity measurements and morphological differences two different solubilization mechanisms for the detergent solubilization of phospholipid vesicles were demonstrated. The first one is a fast solubilization via open bilayer intermediates, which are accompanied by spherical mixed micelles in the coexistence stage and the second one is a slow solubilization of closed vesicles which are accompanied by worm-like micelles. The results presented here fit nicely with the model for the solubilization of phospholipids as proposed by Kragh-Hansen and co-workers [10]. Both here and in the study by Kragh-Hansen a difference between fast and slow solubilization of phospholipids was observed. The fast, transbilayer, solubilization was proposed to occur via open vesicular intermediates as is supported here by cryo-electron microscopic data. Similar open vesicle intermediates have been observed previously by using the same detergents [20–23]. We found no difference between small vesicles obtained by sonication [20,22] and large vesicles [21] obtained by extrusion. The open vesicles were found to be stable for at least several weeks.

The slow, micellar, solubilization is proposed to go via mixed micelles that pinch-off from detergent saturated vesicles. This is supported by the observation of closed vesicles during the entire stage II of the solubilization. It must be mentioned that the presence of closed vesicles is due to non-equilibrium conditions. After prolonged equilibration only worm-like micelles were found in stage II (Fig. 1e). The observation of closed vesicles does not imply that they are not leaky. As to be expected vesicles start to leak when surfactants are incorporated into the bilayer.

In stage II of the solubilization the open vesicular structures of transbilayer solubilization are accompanied by spherical mixed micelles, whereas the closed vesicles from the micellar solubilization are accompanied by worm-like micelles. Also these worm-like micelles have been reported previously upon detergent solubilization of phospholipids both alone [6] and in coexistence with vesicles [24].

This observation brought the idea that the overall packing-parameter, as introduced by Israelachvili [25–27], of the detergent-saturated bilayer determines the solubilization mechanism. For an overview of the structures formed at different packing parameters see Fig. 5. The dimensionless packing parameter is defined as the ratio between the volume of the hydrophobic part of the amphiphiles and the optimal cross-sectional surface area of the hydrophilic head group times the all-trans hydrophobic chain length, $P = V/a_0 \cdot l_c$, were V is the hydrophobic volume, a_0 optimal cross-sectional area and l_c the hydrophobic chain length.

Detergents with a packing parameter $<1/3$ form micelles when there concentration is above the CMC. Double-tailed phospholipids, on the other hand, have packing parameters

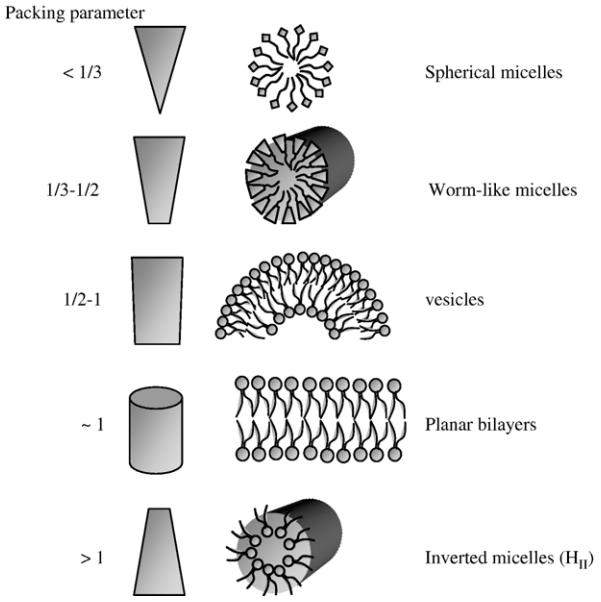

Fig. 5. The packing parameter is determined by the shape of the molecule and determines the aggregated structures that are formed. Redrawn after [26].

around 1 and form bilayers, or packing parameters larger than 1 such as phosphatidyl-ethanolamine which has a high tendency to form inverted hexagonal phases (H_{II}). When detergents are incorporated into the phospholipid bilayer the overall packing parameter will be lowered. Although a_0, V and L_c are not independent parameters [28] and absolute values for the packing parameter of each of the surfactants and of the detergent-saturated bilayer cannot be given, it is nevertheless possible to predict variations of a_0, V and L_c and therefore this approach represents indeed a powerful tool to rationalize and predict mixed detergent phospholipid phase behaviour [29].

The packing parameter of the detergent saturated bilayer, with a detergent–phospholipid combination compatible with the transbilayer model, is probably between 1/2 and 1 resulting in the observed stable open bilayer structures. During stage II of the solubilization, between R_{sat} and R_{sol}, the fraction of open saturated bilayers decreases (decreasing turbidity) whereas the fraction of Triton X-100 DOPC mixed micelles increases upon increasing the detergent concentration.

The packing parameter of the saturated bilayer, with a detergent–phospholipid combination belonging to the micellar model, for which worm-like micelles were observed, is probably between 1/3 and 1/2 and, although it takes time, will completely change from bilayer to worm-like micelles (Fig. 1e). This fits with the observation that Triton X-100 with an ethylene-oxide head group with a relatively small head group cross-sectional surface area (47.6 Å^2, see supporting information) and a large hydrophobic volume (branched, aromatic C_{14}, larger packing parameter) belongs to the transbilayer model, whereas decylmaltoside with a double sugar head group with a relatively large head group cross-sectional surface area (57.0 Å^2, see supporting information) and a smaller hydrophobic volume (linear C_{10}) (smaller packing parameter) belongs to the micellar model.

The composition of the detergent saturated bilayer critically depends on the partitioning of surfactant between solution and bilayer [30,31]. Although R_{sat} for Triton X-100 and decylmaltoside are reached at different concentrations the composition of the bilayer is almost identical due to the large difference in mole-ratio partitioning coefficient [31,32]. For Triton X-100 3.0 and for decylmaltoside 0.2 (10^3 M^{-1}) was found [30]. According to equation (3) in [31] (see supporting information) the bound detergent concentration at R_{sat} is for both detergents around 1.4 mM. Therefore a comparison between the two surfactants is legitimate and the difference in solubilization mechanism could well be due to the overall packing parameter of the detergent-saturated DOPC bilayer.

Changing from βOG to OTG will decrease the packing parameter because the head group cross-sectional area of OTG is slightly larger than that of βOG (oxygen vs. sulphur, βOG, 41.3 Å^2 and OTG, 47.2 Å^2, see supporting information) whereas the volume and the length of the molecule are almost unchanged. Saturation for OTG is reached at almost half the concentration of βOG, but the partition coefficient of OTG is twice as high as that of βOG [30] resulting in a similar bilayer composition. The decrease in packing parameter is apparently enough to change the solubilization mechanism from trans-bilayer to micellar and consequently the packing parameter from above 1/2 to below 1/2. A similar decrease in packing parameter can be achieved by changing the phospholipid composition. When βOG was used to solubilize DMPC a similar shift from trans-bilayer to micellar solubilization was observed. DMPC has, compared to DOPC, a smaller packing parameter because the hydrophobic volume of the molecule is less due to the shorter saturated tails, whereas the length and the surface cross-sectional area (choline) are almost unchanged [33,34]. Another change in solubilization mechanism was observed by using the zwitterionic detergent CHAPS. Here the mechanism could be changed by the addition of NaCl. At a low salt concentration a micellar model was observed, whereas at high salt transbilayer solubilization did occur. This can be explained by a reduction of the surface cross-sectional area (of both the phospholipids and the detergent) upon increasing salt concentration by screening of the electrostatic effect, which will lead to an increased packing parameter. In stage II of the solubilization this will give at low salt worm-like micelles whereas at high salt open bilayers are formed as was reported before [35]. An increase of the overall packing parameter could in this case also be put into effect by an increase of the amount of DOPE, which has a packing parameter larger than 1.

An analogous change in solubilization behaviour was found for the interaction between C_{12}- or C_{16}TAC and egg PC [24]. C_{12}TAC with a relative small packing parameter gives closed vesicles accompanied by wormlike micelles, whereas C_{16}TAC with a somewhat larger packing parameter results in perforated vesicles [24]. Upon decreasing the packing parameter during the solubilization process one would expect to go from bilayers through worm-like structures ending up with spherical micelles. In our study we found only the two different models presented. In some cases this entire process was found in systems with

mixed phospholipids were some domain forming might be involved [36].

During the reverse process, the reformation of bilayers out off mixed micelles, again the packing parameter determines whether bilayer structures or worm-like micelles are formed in stage II. At packing parameters larger than 1/2 bilayer fragments will be formed in the coexisting stage upon detergent removal. The speed of detergent removal and the range of stage II determine the size of the finally formed unilamellar vesicles. If the packing parameter is low (below 1/2) worm-like micelles are formed with a high tendency to bend and coalesce, resulting in the formation of the densely packed multilamellar structures as is shown here for various detergents and as was suggested previously for dodecylmaltoside [6]. When membranes are saturated with detergents a phase separation might occur [37]. In our system with DOPC and decylmaltoside both during solubilization and reformation around R_{sat} stays lamellar even after prolonged equilibration (Figs. 2c and 3c). In the case when OTG was used a phase separation was observed around R_{sat}. However, the behaviour of this system is identical to the behaviour of the decylmaltoside system in stage II of the solubilization and after complete detergent removal.

For the reconstitution of membrane proteins two mechanisms have been proposed. Membrane proteins are either reconstituted during the transition from mixed micelles to proteoliposomes or membrane proteins are incorporated in to detergent-destabilized liposomes [5,38,39]. Membrane proteins can only be reconstituted into bilayers; therefore reconstitution in lipids with a detergent combination that solubilizes via the micellar model is unlikely when the detergent concentration is higher than R_{sat}, because above R_{sat} worm-like micelles are present and no bilayer structures [12]. Phospholipid–detergent combinations that solubilize via the transbilayer route are able to reconstitute membrane proteins over a much wider range, because bilayers are present in the concentration range from R_{sat} to R_{sol}. This is in agreement with results obtained by the group of Poolman, who showed that the lactose transporter LacS could be reconstituted in dodecylmaltoside destabilized liposomes (round R_{sat} when bilayers are still present) or in Triton X-100 solubilized lipids (any where between R_{sat} and R_{sol}) [40]. From our experiments it is clear that dodecylmaltoside solubilizes via the micellar model and thus reconstitution at detergent concentrations above R_{sat} is unlikely. However Triton X-100 solubilizes via the transbilayer model and reconstitution can take place anywhere between R_{sat} and R_{sol}. It must be mentioned that different lipids have different packing parameters and could thus behave differently. Upon reconstituting membrane proteins the protein contributes to the overall packing parameter and could change the solubilization mechanism [12].

As demonstrated before detergents that solubilize via the micellar mechanism do not migrate from the outer to the inner phospholipid leaflet when added externally [10]. The consequence is an excess of molecules in the outer monolayer that is accommodated by a shape change. This is nicely shown for giant liposomes [18] and is demonstrated here for small liposomes by cryo-electron microscopy and fluorescence spectroscopy.

In conclusion, the present results fits with the model presented earlier in which a fast and a slow mechanism are proposed for vesicle solubilization and reformation [10]. However, here we propose that the overall packing parameter of the detergent-saturated bilayer determines which of the two solubilization mechanisms actually occurs under specific conditions.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbamem.2007.06.024.

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