Use of a Dynamic Light Scattering Technique To Study the Kinetics of Liposome Solubilization By Triton X-100 ‖

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Notes

Use of a Dynamic Light Scattering Technique To Study the Kinetics of Liposome Solubilization By Triton X-100[||]

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Introduction

A number of studies have been devoted to elucidate the vesicle to micelle transformation induced by surfactants in phospholipid vesicles using cryo-TEM[1−3] and light-scattering techniques.[4−8] Despite the controversy about the complexity of this transformation, recent studies using vesicles prepared from nonionic and oppositely charged surfactants proposed rapid and simplier mechanisms as single-step processes.[9−13] In a recent paper we reported the solubilization of phosphatidylcholine liposomes by Triton X-100 as a process ruled by direct formation of mixed micelles within the bilayer without formation of complex intermediate aggregates.[14] These findings raise a number of questions about the kinetics of this simple transformation. To clarify these questions, a kinetic study using a dynamic light scattering technique is presented. This technique is based on the use of a Ar laser source, useful in systems in which small and large particles coexist. The complementary use of this technique and freeze-fracture electron microscopy opens up new avenues in the kinetic study of the solubilization of biological membranes by surfactants. Triton X-100 surfactant has been selected given its properties as a good agent for membrane solubilization.[5,15,16]

Materials and Methods

PC was purified from egg lecithin (Merck, Darmstadt, Germany) by the method of Singleton[17] and was shown to be pure by TLC. Triton X-100 (octylphenol polyethoxylated) was especially prepared by Rohm and Haas (Lyon, France) containing an average of 10 ethylene oxide units per molecule and active matter of 100%. Tris(hydroxymethyl)aminomethane (TRIS) was obtained from Merck. TRIS buffer was prepared as 5.0 mM TRIS adjusted to pH 7.4 with HCl, containing 100 mM of NaCl, and was filtered through Millipore membranes type GS 0.22 μm (Bedford, MA). Liposomes of a defined size (of about 200 nm) were prepared by extrusion of large unilamellar vesicles (through 800−200 nm polycarbonate membranes) previously obtained by reverse phase evaporation.[14,18]

To study the kinetics of solubilization of PC liposomes, various T_{X-100} concentrations in TRIS buffer (from 0.1 to 2.0 mM) were added to liposomes (constant PC concentration 0.5 mM). These concentrations were lower and higher than its critical micelle concentration (CMC), which was 0.15 mM, according to the surface tension measurements in the working medium.[19]

The hydrodynamic diameter (HD) of pure PC vesicles, pure T_{X-100} micelles, and particles formed during the interaction of T_{X-100} with liposomes was determined by means of a dynamic light scattering (DLS) technique using a photon correlator spectrometer (Malvern Autosizer 4700c PS/MV) equipped with an Ar laser source (wavelength 488 nm). Quartz cuvettes were filled with the samples, and all of the experiments were thermostatically controlled at 25 °C. DLS determinations were made with a scattering angle of 90° in all cases. To acquire the full range of decay times necessary to determine the signal from both the large and the small particles, a low sample time value (2 μs) and a dilatation of 3 with parallel subcorrelators was used. This allowed for the correlograms to extend up to 1.7 ms.

DLS measurements were performed during the initial 20 s of the mixing (20 s for DLS measurement followed by 10 s for the analysis of data) to study the initial steps of the process. These measurements were continued for 30 min, the minimum needed to obtain statistically reliable data. Measurements from 30 min to 24 h were performed in intervals of 30 min. The analysis of the data was performed using CONTIN software provided by Malvern Instruments, Malvern, England. The goodness of the CONTIN fits was tested by fitting a single or a biexponential to the correlation function. If a biexponential had to be fitted, first a single exponential was fitted to a long time range and then the

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[||] Abbreviations: T_{X-100}, Triton X-100; PC, phosphatidylcholine; DLS, dynamic light scattering; FFEM, freeze-fracture electron microscopy; HD, hydrodynamic diameter; SANS, small-angle neutron scattering.

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second exponential was fitted to the residual. Both methods agreed fairly well. The results are given as diameters and the percentage corresponds to intensity values.

A freeze-fracture electron microscopy (FFEM) study was carried out 20 s after mixing the liposomes with the surfactant to characterize the aggregates formed in the initial interaction steps (first DLS measure). The experiments were done according to the procedure described by Egelhaaf et al.[20] About 1 μL of suspension was sandwiched between two copper platelets using a 400-mesh gold grid as spacer. Then, the samples were cryofixed by dipping into nitrogen-cooled liquid propane at −180 °C and fractured at −110 °C and 5×10^{-7} mbar in a BAF-060 freeze-etching apparatus (BAL-TEC, Vaduz, Liechtenstein). The replicas were obtained by unidirectional shadowing with 2 nm of Pt/C and 20 nm of C, and they were floated on distilled water and examined in a Hitachi H-600AB TEM at 75 kV.

Results and Discussion

First of all size distribution curves for the micellar T_{X-100} solutions before they were mixed with liposomes, and for pure PC liposomes, were obtained. The curve for pure PC liposomes exhibited a monomodal distribution with a HD of 186 nm. The curves for micellar T_{X-100} solutions (T_{X-100} concentration ranging from 0.2 to 2.0 mM) also showed a monomodal distribution with a peak at 9 nm. This value is in line with the reported spherical diameter determined by small-angle neutron scattering (SANS) (about 8 nm).[21,22] The slight difference between these two values is due to the fact that DLS measures the HD, whereas SANS measures the size on the basis of contrast scattering length differences between the particle and the solvent. The kinetic study of the T_{X-100}/liposome interaction was performed using a DLS technique. After 20 s of mixing, one peak for large particles (HD of 196 nm) and another peak for small particles (HD of 17 nm) were detected for 0.1 and 2.0 mM T_{X-100}, respectively. Intermediate amounts of T_{X-100} resulted in two peaks (small and large particles). The small particles corresponded to mixed micelles and the large ones to saturated mixed vesicles (largest particles of 205 nm for 0.3 mM T_{X-100}) or those in the process of disintegration. Increasing T_{X-100} amounts led to a rise and to a fall in the proportion of the small and large particles, respectively. These variations were specially pronounced from 1.5 to 2.0 mM T_{X-100}. Before the formation of mixed micelles starts to occur (sublytic level), the generalized absence of pure T_{X-100} micelles (particles of 9 nm) 20 s after mixing, and the initial growth of mixed vesicles (from 186 to 205 nm), indicated the adsorption of surfactant into bilayers. This finding raises a question about whether the T_{X-100} was adsorbed into bilayers as micelles or as individual monomers. Obviously, in systems below CMC the adsorption is monomeric either through the hydrophilic holes created by these monomers on the PC polar heads or via formation short-lived complexes of surfactant−PC polar heads, in accordance with the studies of Lasic on phase behavior of membranes.[23] As for systems above the CMC, a monomeric adsorption could also be expected, breaking the surfactant micellar structure. This monomeric adsorption may be considered as a two step process: first, the release from micelles to monomers (associated to a micellar relaxation time τ_2[24]), and second, the subsequent incorporation of these monomers into bilayers following the aforementioned mechanisms reported by Lasic.[23] Given that no pure micelles were detected after 20 s of mixing, it was not possible to determine τ_2 in any case. Hence, it was not possible to evaluate whether the rate-limiting step of the adsorption process was associated with the first (micelle to monomer) or second (monomer to vesicle) step.

The formation of mixed micelles requires not only the adsorption of surfactant monomers into bilayers, but also the desorption of mixed micelles from the bilayer surface.[14] The time associated with this desorption was defined as "induction time"[25] and was independent of T_{X-100} concentration. The DLS data for each system measured during the initial 20 s after the surfactant addition are given in Table 1.

The percentages of remaining mixed vesicles vs time for different T_{X-100} concentrations are plotted in Figure 1. The difference with respect to 100% corresponds to the mixed micelles. It may be seen that, using a T_{X-100} concentration lower than its CMC (0.1 mM), the initial mixed vesicles persisted throughout the process (24 h)

Table 1. Particle Size Distributions for PC Liposomes (PC liposomes 0.5 mM) and Different T_{X-100}/PC Systems Varying the T_{X-100} Concentration Measured during the Initial 20 s after the Surfactant Addition to Liposomes[a]

	curve distribution (particle no.) after 20 s of mixing				
		1st peak		2nd peak	
surfactant concn (mM)	type[b]	nm	%	nm	%
0	M			186	100
0.1	M			196	100
0.2	M			204	100
0.3	M			205	100
0.4	B	12.6	4	172	96
0.5	B	13.3	7	170	93
1.0	B	16.0	10	98	90
1.5	B	17.1	33	27.3	67
1.75	B	17.2	69	25.4	31
2.0	M	17.0	100		

[a] Results are given as hydrodynamic diameter, and the percentage corresponds to intensity values. [b] Key: M, monomodal; B, bimodal.

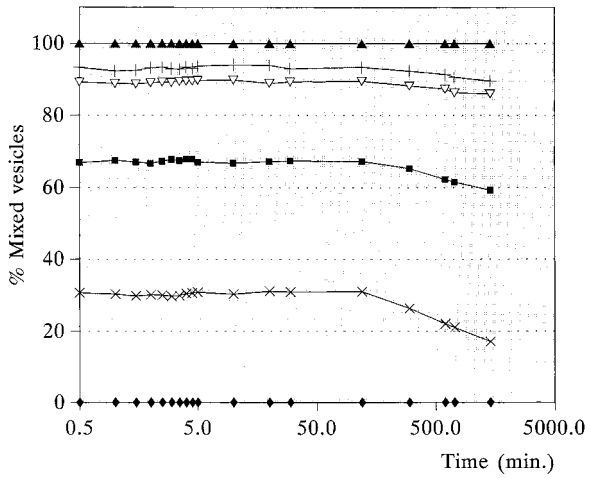

Figure 1. Variation in the intensity percentage of mixed vesicles vs time in the interaction of T_{X-100} at different concentrations with PC liposomes (0.5 mM PC). Surfactant concentrations: (▲) 0.1 mM, (+) 0.5 mM, (▽) 1.0 mM, (■) 1.5 mM, (×) 1.75 mM, and (◆) 2.0 mM.

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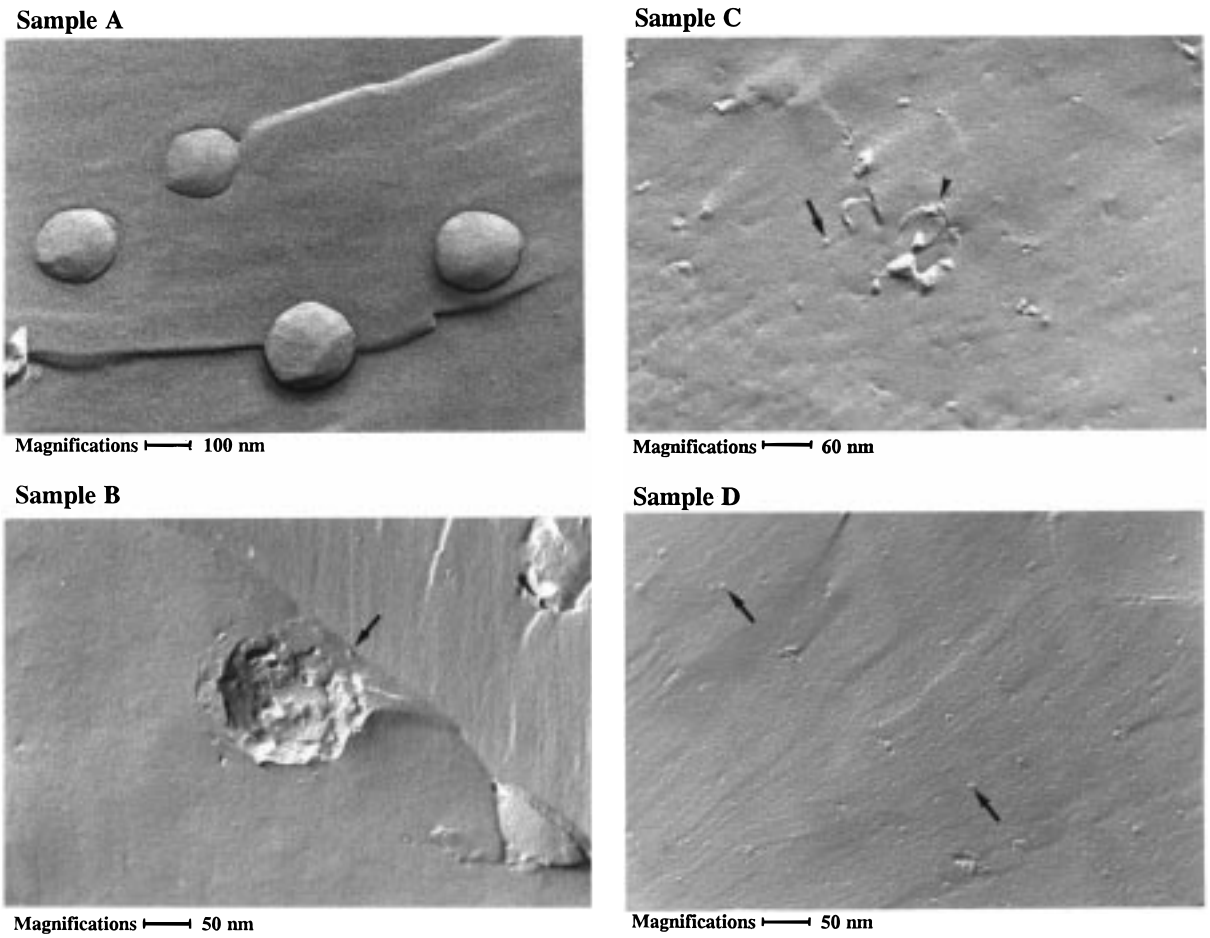

Figure 2. Freeze-fracture electron micoscopy images for pure PC liposomes (sample A) and for three surfactant/PC systems. The T_{X-100} concentration of these systems was 0.5, 1.75 and 2.0 mM, respectively (samples B, C, and D). The PC concentration remained constant (0.5 mM) in all cases. Structures are marked as follows: mixed micelles with arrows and vesicle fragments with arrowheads.

almost without size changes. The dynamic equilibrium between mixed vesicles and mixed micelles achieved during the 20 s after the addition of intermediate T_{X-100} concentrations (from 0.5 to 1.75 mM), also persisted for at least 2 h. Longer periods of time led to a fall in the proportion of large particles (vesicle fragments) and to a rise in that of mixed micelles. These variations were directly dependent on the surfactant concentration in the system. Thus, the percent of mixed vesicles for 1.75 mM T_{X-100} decreased by more than 50% (from 30 to 14%), whereas for 1.5 mM this decrease was only 12−13% (from 66 to 58%). In addition, the mixed micelles (17 nm) formed using the highest T_{X-100} concentration (2.0 mM) persisted throughout the process. To analyze the aggregates formed 20 s after the surfactant addition to liposomes, freeze-fracture electron microscopy was used. Figure 2 shows four representative micrographs for pure PC liposomes (sample A) and for three systems containing 0.5, 1.75 and 2.0 mM T_{X-100} concentration (samples B, C, and D, respectively). Sample A shows typical vesicles. Sample B shows vesicle structures with clear signs of disintegration ("in situ" perforation) and small particles corresponding to mixed micelles (arrows). The micrograph of sample C shows the coexistence of mixed micelles (arrows) and vesicle fragments (arrowheads) without formation of intermediate aggregates. Sample D shows only the presence of mixed micelles (arrows). These observations corroborate the DLS measurements. Furthermore, these images are in line with the observations reported for the same interaction (at the same interaction times) 24 h after the T_{X-100} addition to liposomes.[14] Hence, the structural transformation previously reported already occurred 20 s after mixing.

Comparison of the present information with the kinetics of solubilization of the same PC liposomes with sodium dodecyl sulfate (SDS)[25] shows significative differences. Thus, although the process occurred in both cases, by direct formation of mixed micelles without formation of complex aggregates, the particle size variations and the times needed for these transformations were clearly different. Thus, whereas the sublytic interaction with T_{X-100} occurred with increase in the size of the mixed vesicles, in the case of the SDS, no growth of theses structures was observed. Furthermore, the induction time and the equilibrium time (times needed to reach the equilibrium mixed vesicles−mixed micelles) were clearly lower (half or less) than those needed for the SDS in all cases and were independent of the amount of added surfactant.

The different growths of mixed vesicles may be attributed to the different molecular weight (an average of 620 $g \cdot mol^{-1}$ for T_{X-100} and 288 $g \cdot mol^{-1}$ and SDS) and molecular structure of both surfactants. It is known that T_{X-100} molecule has a hydrophilic region that extends one ethylene oxide chain length about 1.6 nm beyond the hydrophobic core, making the diameter of the whole micelle about 9 nm.[26] These reasons could explain the different micellar sizes (9 nm for T_{X-100} and 4 nm for SDS).

The kinetic variations of both interactions may be attributed to the different electrostatic and structural

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characteristics of both surfactants given the identical characteristics of the liposomes used. The electrostatic interactions (case of the SDS) do not play an enhancing effect on the kinetics of surfactant adsorption into bilayers (understood as a two-step process) and subsequent formation of mixed micelles. However, the hydrophobic surfactant characteristics (hydrophilic−lipophilic balance) seems to be an important factor. In this sense, Kragh-Hansen et al., correlated the flip-flop rate across the lipid bilayer with the hydrophilic/lipophilic properties of the surfactants.[27] Given that T_{X-100} is more hydrophobic than SDS, a faster kinetics with T_{X-100} should be expected if solubilization is dependent on the flip-flop rate.

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