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Effect of Triton X-100 on the physical properties of liposomes

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The interaction of the nonionic detergent Triton X-100 with phospholipid bilayers of liposomes made of egg yolk phosphatidylcholine was studied through the behavior of several physical properties. The dielectric permittivity spectra between 30 kHz and 13 MHz, the viscosity, the density, and the d.c. conductivity (1 kHz) of aqueous liposomes suspensions at various mole ratios were measured at 22°C. For detergent-to-phospholipid ratios lower than 3, a dielectric relaxation process of characteristic frequency of about 50 kHz was recorded. This process does not appear for the liposomes in water, and becomes smaller and smaller for detergent-to-phospholipid ratios higher than 3. The viscosity of these suspensions showed a biphasic behavior, being remarkably increased by the detergent for concentration ratios lower than 3. The measured d.c. conductivity of these samples showed no relation with this process, being slightly increased when the detergent content is increased. As a conclusion of these results a well defined concentration range appears where the phospholipid organization changes forming highly asymmetrical structures.

Introduction

The nonionic detergent Triton X-100 is one of the most effective solubilizers used for isolation and purification of membrane-bound proteins, and reconstitution of lipids and proteins to form functional membranes [1–4]. It has been successfully used for the solubilization of erythrocytes, mitochondria, lysosomes, other biological membranes [2,5–8], and liposomes [9,10].

The primary effect of Triton X-100 on the solubilization of membranes seems to be on the organization of the lipids [2,11–13].

In the present work several physical properties of liposomes suspensions have been used to study the interaction of Triton X-100 with the phospholipid bilayer. The measured changes in the dielectric permittivity spectra indicate a drastic structure transformation into entities of fairly high dipolar moment at a well defined concentration ratio. The measured d.c. conductivity showed no relation with this phenomena, while the viscosity measurements confirm such structure changes. These asymmetrical entities could either be oblate or prolate ellipsoids, having an 'equivalent spherical radius' of 10 nm.

Materials and Methods

Egg phosphatidylcholine (PC) (type V-E) was purchased from Sigma, St. Louis, MO, U.S.A., and Triton X-100 was a donation from Rohm and Haas, Buenos Aires, Argentina, both used without further purification. Water was double destilled of d.c. conductivity lower than 1 μS/cm in all cases. Liposomes suspensions were prepared by consecutive filtration steps as previously described [14], thus having a fairly uniformly sized population of diameter close to 0.6 μm. The respective Triton X-100 concentration was added to the liposomes

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suspension immediately before performing each experiment.

The PC concentration of the samples was determined by measuring the ultraviolet absorption spectra between 200 nm and 300 nm, as suggested by Klein [15].

Dielectric permittivity measurements were recorded using an LF Impedance Analyzer, Hewlett Packard 4192A, equipped with a thermostatted capacitance cell. Viscosity was measured with a digital viscometer Schoott Gerate AVS 300, equipped with a microcell Ubbelohde. Density was measured using a calculating digital density meter Anton Paar DMA46. Conductivity was measured with a conductivity meter Radiometer CMD3, equipped with a thermostatted cell. Ultraviolet spectra were recorded using a spectrophotometer Metrolab 2500.

All measurements were performed at 22°C.

Results

In the dielectric measurements the liquid samples have been exposed to weak electric fields $E(t)$ ($=0.5$ V/cm) varying with time t periodically ($E(t) = E\exp(i2\pi ft)$) with frequency f, for which many discrete values have been chosen between 30 kHz and 13 MHz. The Fourier transforms of the dielectric polarization $P(t)$ induced by $E(t)$ is connected with that of $E(t)$ by the frequency-dependent (relative) complex dielectric permittivity $\varepsilon^*(f) = \varepsilon'(f) - i\varepsilon''(f)$, according to $P(f) = [\varepsilon^*(f) - 1]E(f)/4\pi$.

Fig. 1 shows the measured spectra $\varepsilon'(f)$. The dielectric permittivity of the PC liposomes suspension in water, as well as that of detergent-to-phospholipid ratios higher than 3 is almost constant and always lower than the water permittivity. But, the dielectric spectra of the liposomes treated with Triton X-100, for detergent-to-phospholipid ratios lower than 3, show a completely different behavior. They present a relaxation process at the lower frequency range with a static permittivity well above that of the water. The data scattering below 100 kHz is related to the lower frequency limit of the experimental system. The results for liposomes in water agrees with those obtained by Pottel et al. [16] when the samples were free of ionic impurities.

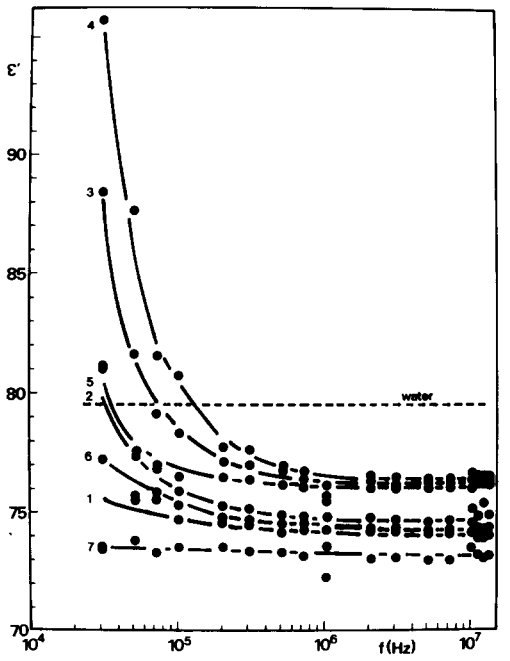

Fig. 1. Real part of the dielectric permittivity ε' with varying frequencies of 1.64 mM PC suspension with different detergent content. Numbers from 1 to 7 indicate the increase of the Triton X-100/PC concentration ratio (C_{TX}/C_{PC}) from 0 to 4.57; 1, 0; 2, 0.76; 3, 1.52; 4, 2.29; 5, 2.74; 6, 3.05; 7, 4.57. The broken line represents the pure water permittivity.

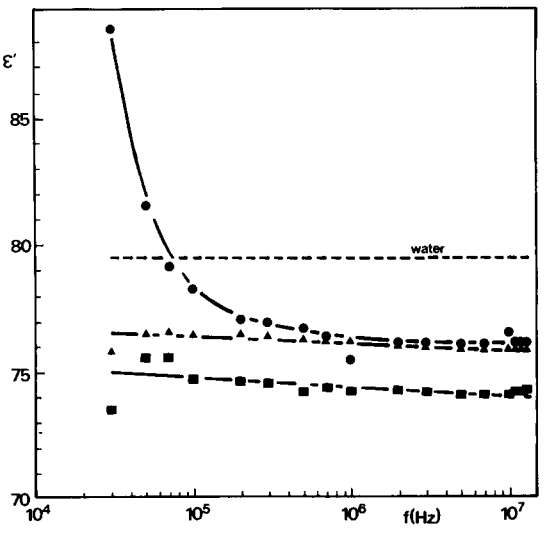

Fig. 2. Comparison of the dielectric permittivity spectra ε'. ●, Triton X-100/PC concentration ratio of 1.52; ▲, 2.5 mM Triton X-100 in water; ■, 1.64 mM PC in water. The broken line represents the water permittivity.

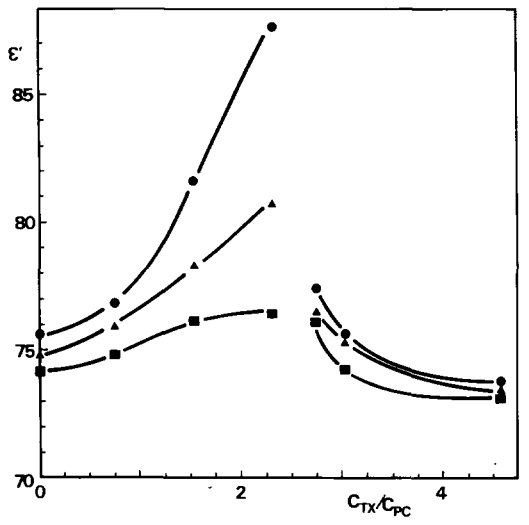

Fig. 3. Dielectric permittivity ε', of the PC suspensions with varying Triton X-100 content, for different frequencies (f): ●, 50 kHz; ▲, 100 kHz; ■, 200 kHz.

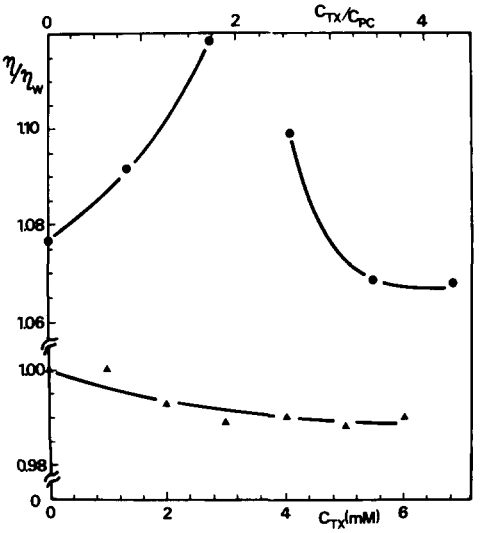

Fig. 5. Viscosity (relative to water) with increasing Triton X-100 concentration: ▲, Triton X-100 in water; ●, 1.64 mM PC suspensions treated with the detergent.

In Fig. 2 the dielectric permittivity spectrum of untreated liposomes is compared with the spectrum of the same sample when the detergent was added, and with that of the respective detergent concentration in water. The spectrum of Triton X-100 in water, as well as that of the PC liposomes, is almost constant. On the contrary, the corresponding spectrum to the sample treated with the detergent presents a relaxation process at the lower frequencies.

In Fig. 3 the dielectric permittivity of phospholipid suspensions with varying Triton X-100 content is plotted for several frequencies. The dielectric permittivity increases with the detergent addition up to a detergent-to-phospholipid ratio equal to 3, in a similar manner for all frequencies plotted.

The increase in the permittivity is not related to the conductivity of these samples, as is shown in Fig. 4. Addition of Triton X-100 to the liposomes slightly increases the conductivity, but in a similar way as it does to the solutions of the detergent in water.

Fig. 5 shows the effect of Triton X-100 on the viscosity. Triton X-100 slightly decreases the viscosity of water. But, it increases the viscosity of phospholipid suspensions in a manner that resembles the behavior of the permittivity (see Fig. 3).

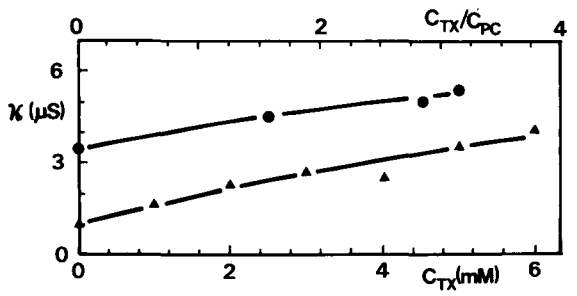

Fig. 4. Direct current (1 kHz) conductivity with increasing Triton X-100 concentration: ▲, Triton X-100 in water; ●, 1.64 mM PC suspensions treated with the detergent.

Discussion

In aqueous solutions, at frequencies considered here, $\varepsilon'(f)$ mainly reflects dielectric polarizability enabled by thermal motion, either rotational orientation of dipoles, or any displacement of electric charges along limited paths. Thus, each dielectric relaxation process can be attributed to several polarization mechanisms that contribute to the total polarizability of the sample.

For conducting media, a rise in the apparent dielectric permittivity at the lower frequency range may be observed, being more remarkable for in-

creasing conductivity. As it is shown in Fig. 4, a monotonic and slight increase in the conductivity is observed when Triton X-100 is added to the liposomes suspension. If the rise registered in the dielectric permittivity (Fig. 1) were due to this effect, then it would show a monotonic increase when the detergent concentration is increased. This is not the case. An inspection of Fig. 3 reveals a remarkable rise of the low-frequency dielectric permittivity, but only within a critical concentration range. For higher detergent concentrations than the critical, where the conductivity is higher, the dielectric effect is not present. Then, this is not related to the conductivity of the samples.

Liposomes used in these experiments are fairly big and weakly polar particles [17]. Thus, their contribution to the total dielectric polarizability, if there is any, must be located at lower frequencies than the range measured in this work. The lowering of the water permittivity by these entiries, thus is due to the water excluded volume (Fig. 2). A similar effect is produced by Triton X-100 (Fig. 2). But, addition of Triton X-100 to the liposomes suspension produces a different effect. A relaxation process is superposed to the lowering of the water permittivity, resulting in a static permittivity well above that of the water. In the high frequency end (10^6–10^7 Hz), the relaxation process still seems to contribute to the dielectric permittivity, although in a very small extent. There, the lowering of the water permittivity is due to the PC and the detergent. Monotonic decreasing values with increasing detergent concentration would be expected at these frequencies, however, inaccuracy prevents any significant conclusion in this sense.

It has been pointed out [2] that addition of detergent to a lipid bilayer produces different phospholipid-detergent complexes, depending on the ratio of the components. The biphasic behavior of the measured viscosity (Fig. 5) reflects this aggregation phenomenon. When small amounts of detergent are added to liposomes, some of it is incorporated into bilayers without disrupting them. As the amount of detergent is increased, the bilayers become saturated with it, and additional detergent induces the formation of mixed micelles [2]. These will have the highest phospholipid content. Previous NMR results [13] indicate that a Triton X-100-to-phospholipid ratio of 2 is required for solubilization of egg PC bilayers of unsonicated liposomes. The maxima observed in both permittivity (Fig. 3) and viscosity (Fig. 5) reported here are consistent with those results. According to Helenius et al. [2], at intermediate detergent-to-phospholipid ratios, bilayers saturated with detergent and micelles saturated with phospholipid coexist in different proportions. However, when enough detergent is added, all the phospholipid is converted to the mixed micellar form, thus producing a clear isotropic solution (concentration ratios between 2 and 2.5). For the saturated mixed micelles of Triton X-100 and sphingomyelin, Yedgar et al. [11] proposed an oblate ellipsoid model which is detergent-enriched in the parts of higher curvature. The measured changes in the dielectric permittivity (Fig. 3) and the viscosity (Fig. 5) suggest that some asymmetrical structures, which may be similar to the model proposed by Yedgar et al., are formed when Triton X-100 is added to the PC liposomes at the corresponding concentration ratios. Additional detergent will then cause an increase in the detergent-to-phospholipid ratio in the mixed micelles, thus decreasing the degree of asymmetry and the size (concentration ratios greater than about 2.5 in Fig. 3 and Fig. 5), as they become more similar to the smaller and more spherical micelles of Triton X-100 [11].

From the dielectric relaxation process observed (Fig. 1), a critical frequency of about 50 kHz was estimated. If the shape and the axial ratio were known, the corresponding relaxation time could be used to determine the size, via the corrected Perrin's formula [18]

$$\tau = 4\pi\eta a^3\rho^2/kT\,; \quad \rho \equiv b/a$$

where η is the viscosity of the solution, T is the absolute temperature, k is the Boltzmann's constant, a and b are the semi-axis of an ellipsoid of revolution, and τ the orientational relaxation time of a sphere equal in volume to the ellipsoid. Unfortunately, neither the shape nor the axial ratio are known. Assuming spherical shape, an 'equivalent spherical radius' of 10 nm is obtained. Computations done for ellipsoidal shapes, for different axial ratios showed that any shape from spherical to highly asymmetric ellipsoids (up to an axial ratio of 3 for oblate and 4 for prolate) is

possible within a reasonable length of the minor axis. This shows that the experimental information at hand is not enough for an unambiguous assignment of size and shape. However, the dielectric permittivity (Figs. 1 and 3), and the remarkable correlation between this and the viscosity (Fig. 5), indeed show an evidence of geometric asymmetry. Triton X-100 and phosphatidylcholine molecules have both a weak polarity. The contribution to the polarization of spherical micelles as a whole, would be almost negligible due to the symmetry, even for a very weak sterical hindrance of the polar heads. Nevertheless, residual ions in the solution could produce a Maxwell-Wagner effect, also for a spherical shape. If the observed polarization were due to this effect, the amplitude of the relaxation process would indicate that the spherical entities grow in approaching the critical concentration, and then become smaller for increasing detergent content. Accordingly, the relaxation spectrum would be shifted from higher to lower frequencies in approaching the critical detergent-to-phospholipid ratio, and then again to higher frequencies. Fig. 1 shows that this is not the case. Instead, the effect seems to grow and vanish without any frequency shift.

We can conclude that the observed polarization may be either due to residual ions in the solution or to the polar heads (presumably in a cooperative effect). But, for any of these mechanisms, highly asymmetric micelles, either prolate or oblate, must be the responsible entities for the observed polarization. For a prolate shape, a major semi-axis of 25 nm is found, for the maximal asymmetry consistent with a reasonable length of the minor axis. For an oblate shape, this is 12.5 nm. To decide unambiguously the shape and degree of asymmetry, some additional information must be available.

Acknowledgements

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