LYSIS AND REASSEMBLY OF SONICATED LECITHIN VESICLES IN THE PRESENCE OF TRITON X-100

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1. Introduction

Sonicated lecithin vesicles are known to give rise spontaneously to larger structures [1–3]. This process was originally interpreted as vesicle fusion, and liposomes have since been recognized as potential models for membrane fusion studies [4,5]. However, more precise definitions of the expression 'membrane fusion', as well as a critical appraisal of the available experimental evidence, have brought into this field the distinction between 'fusion' and 'cracking and reannealing' of membranes [6,7]. The main difference between these two processes would be the transfer of vesicle contents without spillage, that would only happen in true fusion.

On the other hand, the mechanism of membrane solubilization by detergents is an important research field because of the implications of surfactant action on cell fusion [8], intrinsic protein reconstitution, and other interesting areas (review [9]). Liposomes have been used as simple models for the study of membrane solubilization by detergents [10].

This paper deals with the effect of Triton X-100 on sonicated and non-sonicated lecithin vesicles. The detergent greatly enhances the spontaneous tendency of the sonicated vesicles to form larger structures.

2. Materials and methods

Egg-yolk lecithin (EYL) was prepared according to [11]. 1,2-Dimyristoyl-*sn*-glycero-3-phosphorylcholine (DMPC), dicetylphosphate (DCP) and Triton X-100 (Rohm and Haas) were purchased from Sigma (Norbiton Yard, Surrey).

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The lipid mixtures (phosphatidylcholine:DCP, 10:1 molar ratio) were prepared by dissolving the dry lipids in chloroform. A fraction of the solution containing 11 μmol lipid was evaporated to dryness and the lipid resuspended in 5 ml 0.15 M NaCl, 0.0067 M phosphate buffer (pH 7.4). When required, the liposome suspension was sonicated in an MSE sonicator at 10–12 μm amplitude, for 5 min, at a temperature above the transition temperature of the mixture. Triton X-100 was then added to aliquots of the vesicle suspension at 0.00125–1.25% (w/v) final conc. (~40:1 and 1:25 lipid:detergent molar ratios) and the various samples were left at room temperature for 30 min unless otherwise stated.

Turbidity was measured against pure buffer in a DB-GT Beckman spectrophotometer at 500 nm. Release of vesicle contents (potassium chromate) was measured as in [12]. Electron microscopy observations were carried out on freshly prepared samples as follows: A formvar-coated grid was put on top of a drop of liposome suspension for 3 min. After eliminating the excess liquid, the grid was transferred onto a drop of 3% ammonium molybdate, and left for another 3 min. The excess liquid was again eliminated, and the grid observed under a Philips EM-300 electron microscope, at 80 kV.

3. Results

The turbidity of non-sonicated liposome suspensions decreases with increasing Triton X-100 concentrations, as shown in fig.1a. The originally turbid suspension becomes optically clear at ~0.125–0.185% (w/v) detergent. The behaviour of sonicated vesicles is different: slightly opalescent when pure, the addition

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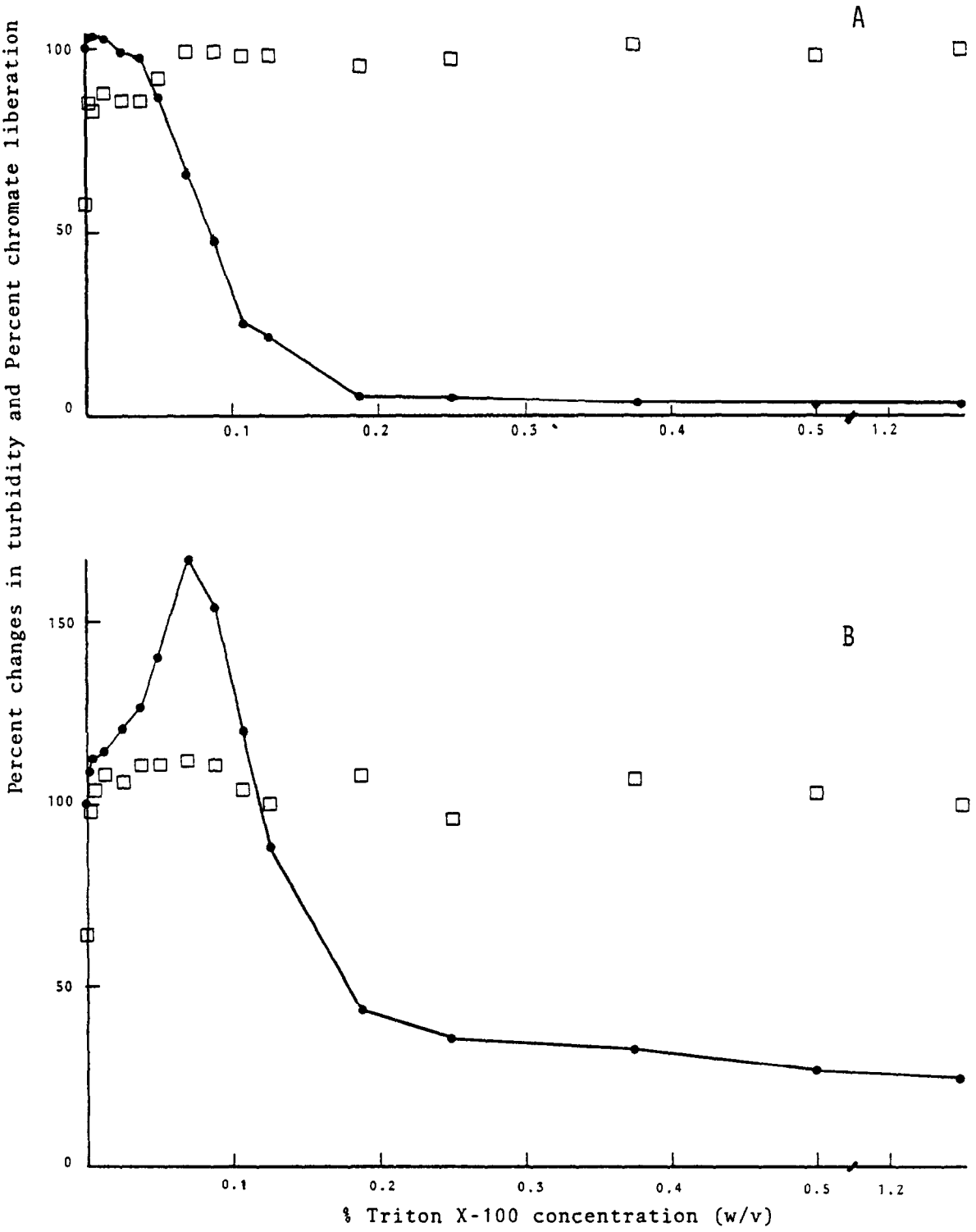


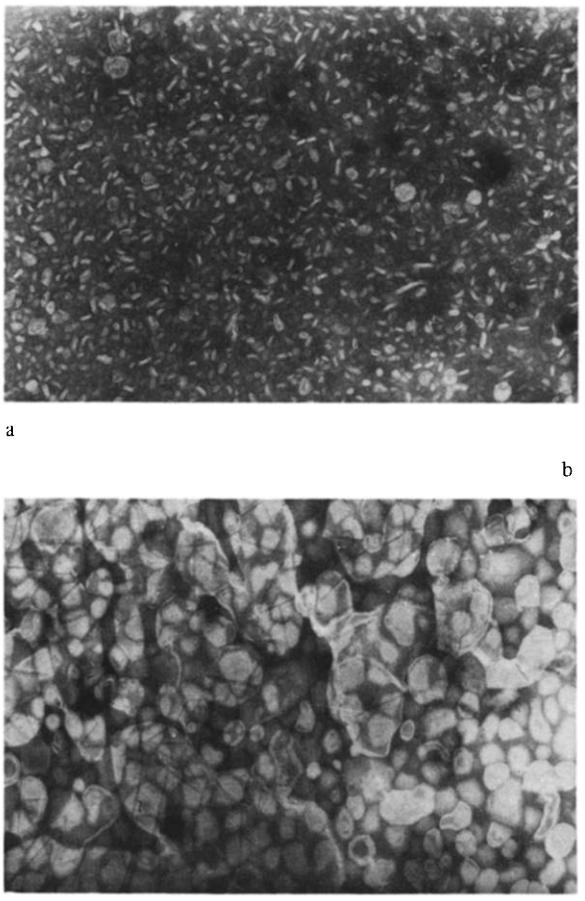
Fig.1. (•) Percent changes in turbidity (i.e., A_{500}) of an EYL–DCP liposome suspension in the presence of various concentrations of Triton X-100 (100% = turbidity in the absence of detergent). (□) Percent chromate liberated from the lipid vesicles by the detergent (100% = chromate liberated at the highest detergent concentration). (A) Non-sonicated vesicles; (B) sonicated vesicles.

of Triton X-100 makes the suspension become more turbid up to a certain point, corresponding to ~1 detergent molecule/lipid molecule (0.068% (w/v) detergent) (fig.1b). The addition of more Triton X-100 causes the turbidity to decrease, until the suspension becomes optically transparent. The ~1:1 stoichiometry for the maximum turbidity is observed for preparations of pure EYL, pure DMPC and phosphatidylcholine:DCP (10:1 molar ratio) mixtures.

With sonicated, as well as with non-sonicated vesicles, chromate liberation is already maximal at the lower detergent concentrations studied (fig.1a,b,□). This means that an important breakdown of membrane architecture, transient or permanent, is produced even by these low detergent concentrations. The amount of chromate 'liberated' in the absence of detergent corresponds actually to the sum of chromate liberated by passive diffusion plus outer chromate not eliminated by the dialysis procedure.

The increase in turbidity of the sonicated vesicle suspensions could be due to reorganization of the lipid material into larger structures. This hypothesis was confirmed by electron microscopic observations (fig.2). A remarkable and gradual increase in size was observed in the case of the sonicated vesicles, parallel to the increase in turbidity. Fig.2b corresponds to the apparent maximum size, and also to the detergent concentration giving peak turbidity. Higher Triton X-100 concentrations did not lead to any change in vesicle size, but it can be said, on a semi-quantitative basis, that the number of vesicles decreased, until virtually no vesicles were left at detergent:lipid molar ratios of ~10:1. Triton X-100 did not produce any appreciable change in the size of non-sonicated vesicles.

To study the effect of lipid fluidity on the process of vesicle growth, vesicles of pure DMPC were prepared above the T_c transition temperature of the lipid (T_c = 23°C [13]). Sonicated and non-sonicated vesicles were then incubated at various temperatures and treated with Triton X-100. Turbidity measurements of vesicles incubated well above and below T_c (30°C and 15°C, respectively) are shown in fig.3. It can be clearly seen that Triton X-100 induces an increase in vesicle size only in the case of sonicated vesicles incubated above, but not below T_c. Turbidity results were confirmed by electron microscopic observations.



a

b

Fig.2. Electron micrographs of a sonicated vesicle suspension (a) in the absence, and (b) in the presence of Triton X-100 at a detergent:phospholipid 1·1 molar ratio. Plate magnification· 53 333×.

4. Discussion

The fusogenic character of amphiphilic substances was first demonstrated in [8,14,15]. The early assumption of spontaneous DML vesicle 'fusion' [1,2] was later modified showing that traces of free myristic acid were responsible for the process of vesicle growth [5].

The above results show that the non-ionic detergent Triton X-100 effectively enhances the growth of sonicated, but not of unsonicated vesicles. It has been shown that sonicated lecithin vesicles have structural defects [16]. The metastable character of sonicated phosphatidylcholine vesicles above the transition temperature T_c was noted in [17]. The 'fusogenic' effect of Triton X-100 is only seen above T_c. In [2,3] highest rates of lecithin vesicle fusion were found at temperatures near T_c of the pure lipid. We have not undertaken any experiments in the T_c region, because of

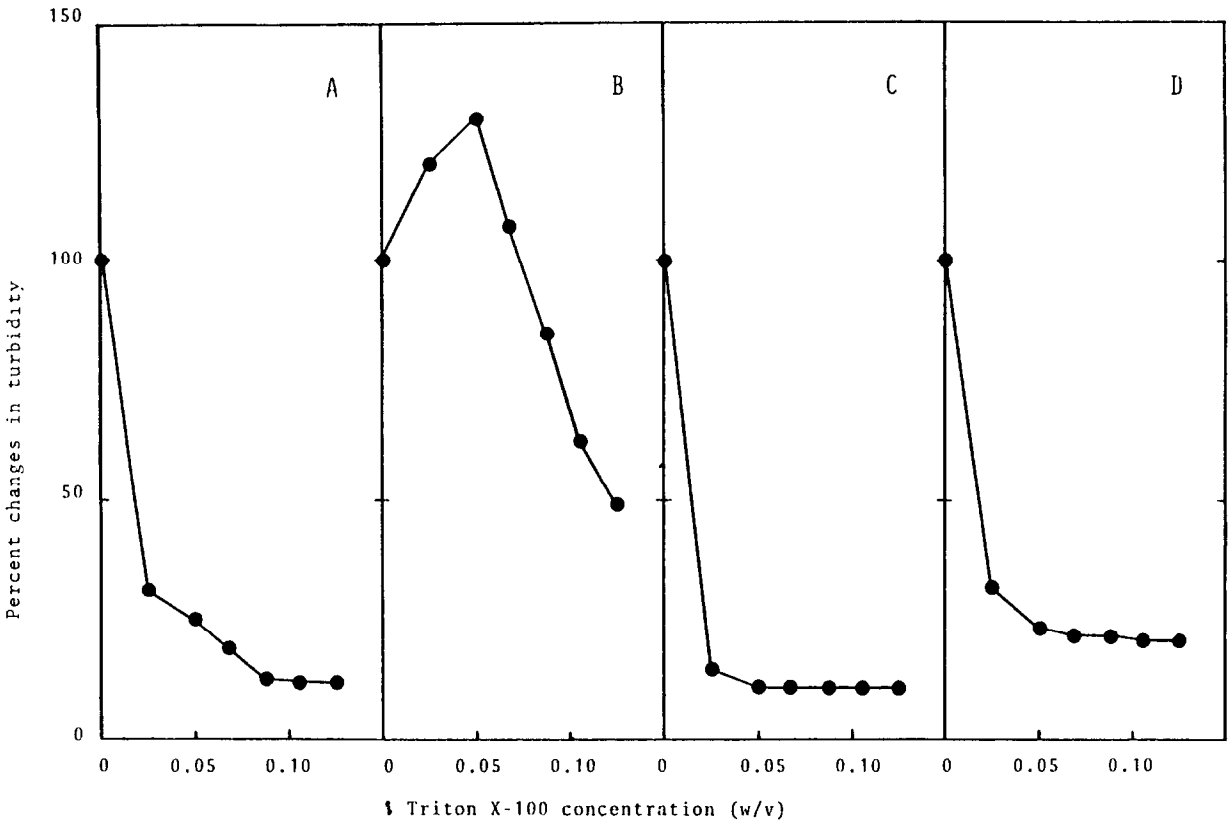


Fig.3. Percent changes in turbidity (i.e., A_{500}) of a DML liposome suspension in the presence of various concentrations of Triton X-100 (100% = turbidity in the absence of detergent). (A) Non-sonicated vesicles, 30°C; (B) sonicated vesicles, 30°C; (C) non-sonicated vesicles 15°C; (D) sonicated vesicles 15°C.

the uncertainties of the transition temperature of sonicated vesicles [18]. T_c can also be modified by the presence of Triton X-100.

Our interpretation of the facts is as follows: Triton X-100 binds to the lipid bilayer at sub-lytic concentrations [19,20]. This detergent acts as a 'wedge' disrupting the bilayer structure [21]. It is conceivable that this wedge effect will liberate the lateral strain imposed on the sonicated vesicles above T_c by the packing faults. The detergent will also tend to bring about a transition from pure lipid in a lamellar phase to lipid–detergent mixed micelles [9]. From then on, a competition will exist between the tendency of the micelles to become solubilized and the tendency of the strain-free open vesicles (containing some bound Triton X-100) to give rise to larger structures. This tendency of the open lamellae to reassembly would be favoured by the fact that these lamellae contain some detergent bound and, presumably, some mixed micelles embedded in the bilayer. The role of micelles in membrane fusion has been pointed out repeatedly [6,8]. Apparently, the prevailing tendency at detergent:lipid molar ratios $<1:1$ is the increase in vesicle size and turbidity, whereas at higher ratios bilayer solubilization prevails, and turbidity decreases. The non-sonicated, strain-free vesicles would not grow, but just participate in the lamellar–micellar transition characteristic of bilayer solubilization.

It is not possible to deduce from these data whether the process of vesicle growth corresponds to membrane 'cracking and reannealing' or to 'true fusion', i.e., whether the vesicle contents are spilled out or not in the process. The uncertainty comes from the fact that the detergent increases enormously the permeability of sonicated and non-sonicated liposomes at concentrations $\geq$50-times smaller than required for vesicle growth, so that virtually all the vesicle contents are liberated by the detergent independently of any

process of vesicle growth. Therefore it is concluded that Triton X-100 induces the reassembly of sonicated lecithin vesicles into larger structures, although it cannot be said whether this happens or not through a fusion process.

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