The effect of bilayer order and fluidity on detergent-induced liposome fusion

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Surfactants induce fusion (or increase in size) of sonicated liposomes. This phenomenon is enhanced by cholesterol and inhibited by the intrinsic polypeptide gramicidin A. By comparison with previous physical studies we conclude that liposome 'fusion' is facilitated when both fluidity and static order of the bilayer are high.

Membrane fusion *Liposome* *Surfactant* *Membrane order* *Membrane fluidity*

1. INTRODUCTION

The increase in size, due to various agents, of sonicated unilamellar liposomes, has been often considered as a model for membrane fusion [1–3]. We have shown [4–8] that many surfactants are able to induce an increase in liposome size, and the similarities between this phenomenon and the physiological events of membrane fusion have been discussed. In one of our earlier papers [4], we pointed out that detergent-induced liposome 'fusion' took place only above the T_c transition temperature of the pure phospholipid. The main thermotropic transition of saturated phosphatidylcholine liposomes brings about a change in both static order and fluidity (or dynamic order) or the bilayer [9]. The variations of both parameters may be independently observed by the appropriate physical techniques: fluidity may be measured by fluorescence polarization of DPH (1,6-diphenyl-1,3,5-hexatriene) or other suitable probes, whereas static order is usually related to the quadrupolar splitting of ^{2}H-NMR spectra [10].

The aim of the present study was to induce variations of order and fluidity as independent as possible from each other, and thus distinguish between the influence of both parameters on detergent-induced liposome fusion. For that purpose, increase in size was observed in liposomes containing either cholesterol or the intrinsic polypeptide gramicidin A.

It is known that, well above T_c, cholesterol increases order, while intrinsic polypeptides do not change, or decrease, this parameter [10–13]; fluidity is only slightly affected under these conditions [14–20]. Gramicidin A is particularly useful as a polypeptide in this study, since it is very easily accommodated in fluid dimyristoylphosphatidylcholine bilayers. Our results provide new information on the mechanisms of liposome 'fusion', and may be relevant to the fusion of cell membranes.

2. MATERIALS AND METHODS

1,2-Dimyristoyl-*sn*-glycero-3-phosphorylcholine (DMPC) and Triton X-100 were purchased from Sigma. Gramicidin A was supplied by Koch-Light. Lipid-lipid and lipid-polypeptide mixtures were prepared by mixing chloroform solutions of the pure components; the resulting solutions were vacuum-evaporated for at least 2 h and resuspended in 0.15 M NaCl, 0.0067 M phosphate buffer

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(pH 7.4), to a 0.0022 M final concentration of phospholipid. Liposome suspensions were sonicated in an MSE sonicator at 10–12 μm amplitude, for 30 min, at a temperature well above the transition temperature of the phospholipid (T_c = 23°C) and centrifuged at 150000 × g at 37°C in order to eliminate probe debris. Aliquots of the sonicated vesicles were treated with equal volumes of the corresponding Triton X-100 solutions in the above-mentioned buffer for 30 min at room temperature. Turbidity was measured as absorbance of the samples at 500 nm in a UV 5260 Beckman spectrophotometer; for practical purposes; ΔA_{500} between detergent-treated and untreated liposome suspensions was considered.

3. RESULTS

Sonicated liposomes of DMPC above their T_c transition temperature show an increase in turbidity when treated with Triton X-100 (fig.1). With pure DMPC, maximum turbidity occurs at about 0.05% (v/v) Triton X-100. This increase in turbidity has been correlated with an increase in size, or 'fusion', of the sonicated vesicles [4,5]. When cholesterol is present in the lipid bilayers, larger changes in turbidity are apparent above T_c (fig.1); maximum ΔA_{500} is found in all cases at the same surfactant concentration. The turbidity maxima increase approximately linearly with cholesterol molar fraction in the lipid bilayer (fig.2). A similar increase is found when the quadrupolar splitting ($\Delta\nu_Q$), an order parameter, of ^{2}H-NMR spectra from selectively deuterated (in the fatty acid methyl end) DMPC bilayers is plotted versus cholesterol molar fraction. The NMR data, taken from Jacobs and Oldfield [12] are also included in fig.2.

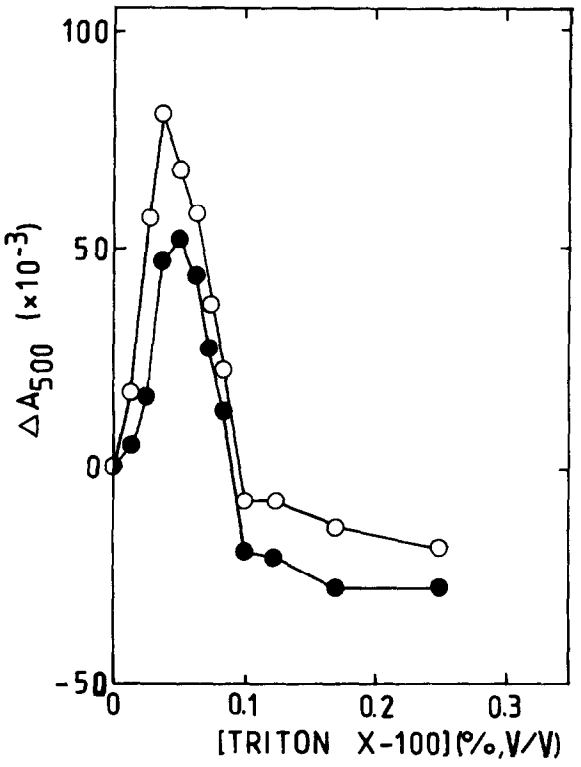

Fig.1. The change in turbidity (ΔA_{500}) of liposome suspensions upon addition of Triton X-100, versus detergent concentration, at 30°C. (●) Pure DMPC; (○) DMPC:cholesterol, 10:1.

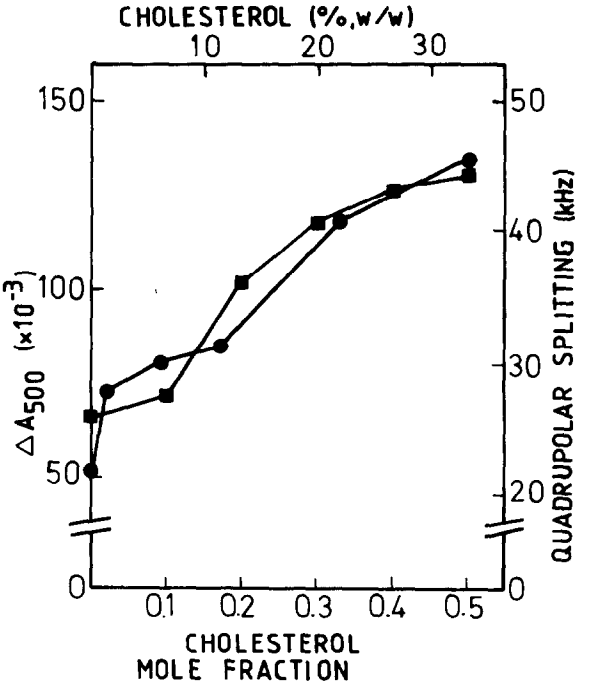

Fig.2. Effects of increasing proportions of cholesterol in the lipid bilayer. (●) The maximum turbidity of detergent-treated liposome suspensions as a function of cholesterol molar fraction; (■) quadrupolar splitting ($\Delta\nu_Q$) of ^{2}H-NMR spectra from deuterated DMPC bilayers containing various proportions of cholesterol. NMR data are taken from [12].

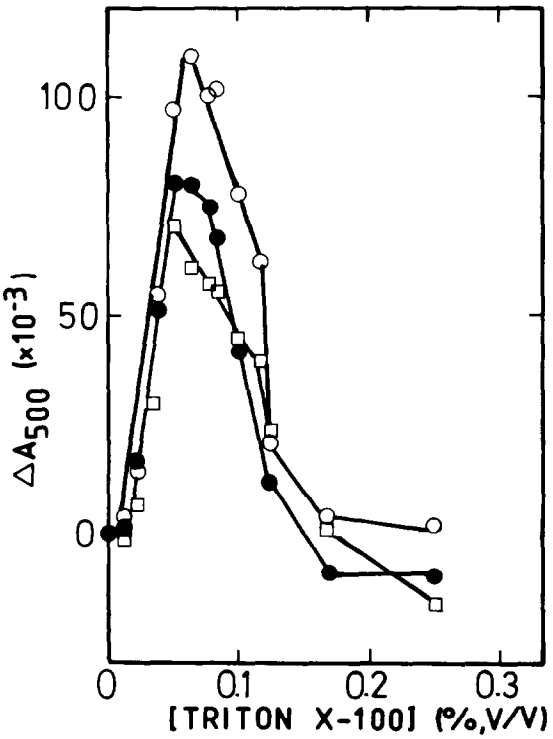

Fig.3. The change in turbidity (ΔA_{500}) of various liposome suspensions upon addition of Triton X-100, versus detergent concentration, at 37°C. (●) Pure DMPC; (○) DMPC:gramicidin A, 500:1; (□) DMPC:gramicidin A, 20:1.

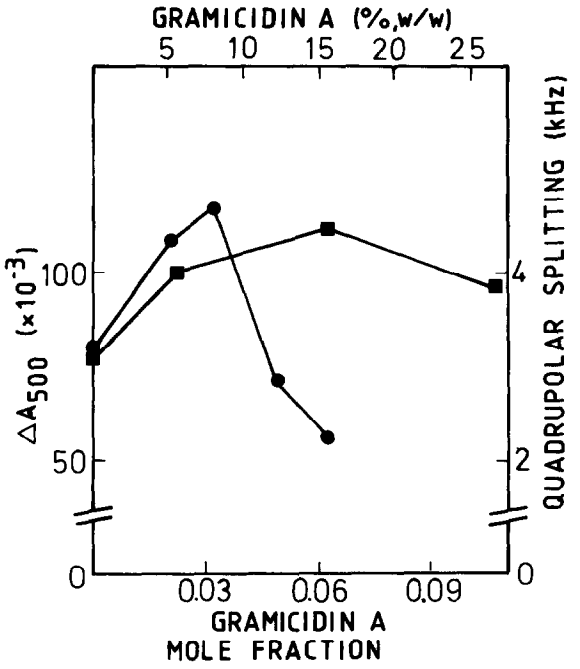

Fig.4. Effects of increasing proportions of the intrinsic polypeptide gramicidin A in the lipid bilayer. (●) The maximum turbidity of detergent-treated liposome suspension as a function of gramicidin A molar fraction; (■) quadrupolar splitting ($\Delta\nu_Q$) of ^{2}H-NMR spectra from deuterated DMPC bilayer containing increasing proportions of gramicidin A. NMR data are taken from [13].

The effect of gramicidin A, an intrinsic polypeptide, on the liposome increase in size induced by Triton X-100 is shown in fig.3. The effect of gramicidin A is more complex than that of cholesterol, since at low polypeptide concentrations (less than one polypeptide per 32 DMPC molecules) ΔA_{500} increases with gramicidin A molar fraction, whereas a decrease in maximum turbidity is observed at higher polypeptide concentrations. The maximum turbidities are plotted vs gramicidin A molar fraction in fig.4. A similar behaviour, with a maximum at 15% gramicidin A, is found for the quadrupolar splitting of ^{2}H-NMR spectra from selectively deuterated DMPC bilayers containing various proportions of gramicidin A, also shown in fig.4. The NMR data have been taken from Rice and Oldfield [13].

When sonicated unilamellar DMPC liposomes are treated with Triton X-100 at temperatures below T_c, bilayer solubilization occurs, and turbidity decreases monotonically with increasing surfactant concentrations: liposome fusion does not occur [4]. Neither gramicidin A nor cholesterol modify in general this pattern; only liposomes containing high amounts of cholesterol (e.g., equimolar ratios) are fused in the presence of detergent below T_c (fig.5).

4. DISCUSSION

The above results show that detergent-induced fusion of sonicated unilamellar liposomes is influenced by the physical state of the lipid, as well as by the presence of cholesterol and the intrinsic polypeptide gramicidin A. In general, liposome fusion (or increase in size) occurs only when the lipid is above T_c, i.e., in the liquid-crystalline state, it is enhanced by cholesterol and low gramicidin A con-

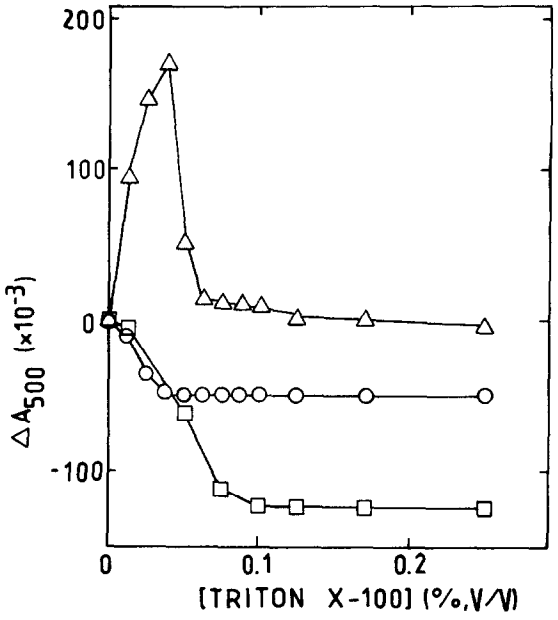

Fig.5. The change in turbidity (ΔA_{500}) of various liposome suspensions upon addition of Triton X-100, versus detergent concentration, at 10°C. (□) DMPC:gramicidin A, 10:1 molar ratio; (○) DMPC:cholesterol, 10:1 molar ratio; (△) DMPC:cholesterol, 1:1 molar ratio.

centrations, and inhibited at DMPC:gramicidin A molar ratios lower than 32:1. These results may be easily understood in terms of lipid fluidity and static order.

Fluidity is a dynamic property of phospholipid bilayers [9,10]. The lateral diffusion coefficient of bilayer components D, is useful in order to evaluate bilayer fluidity. In the liquid-crystalline state, D values obtained for lipids and small peptides through a variety of methods are in the region of 10^{-8} cm$^2 \cdot$s^{-1}; below T_c, D values decrease dramatically by two or three orders of magnitude [19,21]. Above T_c, cholesterol has almost no effect on the dynamics of the membrane; below T_c, cholesterol concentrations up to 10:1 phospholipid:sterol molar ratio have also little effect on the lipid diffusion coefficients. Equimolar lecithin:cholesterol mixtures are known to be in a fluid state ($D \simeq 10^{-8}$–10^{-9} cm$^2 \cdot$s^{-1}) both above and below T_c [9,14–16]. With respect to the effect of small intrinsic polypeptides on lipid fluidity, studies of the lateral diffusion of a modified phosphatidylethanolamine in the presence of gramicidin S reveal that the lipid diffusion coefficient is only reduced by a factor of 1.5 in the presence of 20 mol% gramicidin S [15]. Larger proteins also appear to modify but slightly lipid fluidity [20]. In the light of these studies, it may be said that, except in the case of high-cholesterol containing mixtures, the main change in fluidity occurring in our systems is brought by the thermotropic phase transition. Only above T_c are our liposomes in a relatively fluid state and only above T_c does detergent-induced fusion occur (fig.1,5). Therefore, the fluidity of the liquid-crystalline state appears to be a prerequisite for fusion to occur, irrespective of liposome composition. The increase in size of liposomes containing 1:1 DMPC:cholesterol below T_c (fig.5) confirms this assertion.

Static order is a structural parameter, related to the proportion of *gauche* rotamers in the phospholipid hydrocarbon chains [10]. Deuterium quadrupolar splitting is an 'order parameter' [10–13] that can be obtained from the ^{2}H-NMR spectra of selectively deuterated phospholipids. Oldfield and co-workers [12,13] have studied the effect of cholesterol and gramicidin A on the quadrupolar splitting ($\Delta\nu_Q$) of DMPC deuterated in the fatty acid methyl ends. Cholesterol produces a large increase in $\Delta\nu_Q$ [12], which is in agreement with other measurements based on a pulsed NMR method [14]. The change in quadrupolar splitting produced by gramicidin A is more complex: $\Delta\nu_Q$ is slightly increased by polypeptide concentrations up to 15:1 DMPC:gramicidin A molar ratio, and then decreases, reaching a value near zero at about 3:1 [13]. Larger proteins seem to produce mainly a decrease in quadrupolar splitting. We have replotted the variation of the DMPC order parameter as a function of cholesterol (fig.2) and gramicidin A (fig.4) concentration in the bilayer, together with the maximum turbidity produced by the detergent in each liposome preparation. Turbidity is related to the extent of vesicle fusion [4]. It is clear that detergent-induced increase in size of liposomes follows closely the patterns of change of static order in the bilayer. In the case of gramicidin A, the maxima do not correspond exactly (fig.4); this may be due, among other causes, to the fact that the ^{2}H-NMR experiment shown is just giving information on the fatty acid end, whereas the per-

turbation caused by gramicidin A extends to most carbon atoms in the chain. These results suggest that liposome fusion is facilitated by an increased phospholipid order in the bilayer. The effects of order and fluidity may be easily compared in the case of pure DMPC and 1:1 DMPC:cholesterol liposomes above T_c. Lateral diffusion coefficients are similar in both cases [10], but static order is much higher in the presence of cholesterol [12]; fusion is equally increased when the sterol is present (fig.2). Consequently, for a given fluidity, fusion increases with phospholipid static order. In connexion with our studies, it is interesting to note that plasma membranes, which are involved in many fusion events (secretion, exocytosis, neurotransmitter release) are characteristically rich in cholesterol and poor in proteins [22]. Also, it was pointed out long ago that protein-free regions are required for cell-membrane fusion to occur [23]. On the other hand, the enhancing effect of low gramicidin concentrations on liposome fusion (and bilayer static order) may be understood in the light of recent work suggesting the role of hydrophobic polypeptides in membrane fusion [24,25].

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