THE INTERACTION OF DETERGENTS WITH PHOSPHOLIPID VESICLES

A spectrofluorimetric study

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1. Introduction

Some detergents induce a remarkable increase in size of sonicated liposomes above the main T_c transition temperature of the pure lipid [1,2]. In view of the proposed implications of amphiphilic molecules in membrane fusion [3] it is tempting to speculate that our observations and those of cell fusion might share a common molecular mechanism. In our model system, vesicle growth is accompanied by extensive release of vesicle contents, that is incompatible with true fusion, but it is possible that in the physiological case the amphiphile is acting only on a localized membrane region, and so fusion takes place through the same mechanism, but without massive loss of vesicle contents.

A previous fluorimetric study of the interaction of cell membranes with the so-called fusogenic lipids [4] showed that the latter produced an increase in the fluidity of the membrane lipids. We have shown [2] that some cell fusogens, such as lysophosphatidylcholine or glyceryl mono-oleate, are not effective in promoting any increase in size of phosphatidylcholine liposomes, while the latter grow in the presence of detergents, such as Triton X-100, sodium dodecylsulphate (SDS), sodium cholate or β-octylglucoside. This work was undertaken with the aim of exploring the interaction of various amphipathic molecules with the lipid bilayer, in relation to their ability to promote vesicle growth. The data seem to indicate that, as revealed by spectrofluorimetric techniques, the amphiphiles inducing an increase in liposome size behave in the same way as the fusogenic lipids interacting with cell membranes.

Abbreviations: SDS, sodium dodecylsulphate; ANS, 1-anilino-8-naphthalene sulphonate; NPN, *N*-phenyl-1-naphthylamine; EYL; egg-yolk lecithin

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2. Materials and methods

The details of liposome preparation and detergent treatment have been described in [1,2]. Essentially, egg-yolk phosphatidylcholine (EYL) liposomes were prepared in a 0.15 M NaCl 6.7 mM phosphate buffer (pH 7.4) at 10^{-3} M phospholipid, unless otherwise stated, and sonicated under conditions producing mostly single-shelled vesicles. Aliquots of the liposome suspension were treated with equal volumes of the adequate detergent solutions in the same buffer. After allowing equilibration to take place for 30 min, the intrinsic fluorescence of the preparations was measured under suitable conditions, according to the probe to be used.

1-anilino-8-naphthalene sulphonate (ANS) (Sigma) and *N*-phenyl-1-naphthylamine (NPN) (Aldrich) were used without further purification. Stock solutions in absolute ethanol were prepared daily. ANS (final conc. 20 μM, unless otherwise stated) or NPN (final conc. 3 μM, i.d.) were added to the liposome-detergent systems and left to equilibrate for 1 h at 25°C before carrying out the measurements. These conditions correspond to lipid:probe molar ratios of 25:1 (ANS) and 166:1 (NPN), respectively. Fluorescence measurements were performed in a Perkin Elmer spectrofluorimeter model MPF-3 at 25°C. The extrinsic fluorescence of ANS and NPN were excited at 388 nm and 340 nm, respectively, and the emission

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intensities were observed at 474 nm and 410 nm, respectively, except for the experiments involving emission wavelength variation. Appropriate blanks were run on each occasion, and the intrinsic fluorescence of the system subtracted, although it was usually negligible.

3. Results

The 6 amphipathic substances considered here apart from phosphatidylcholine, namely Triton X-100, SDS, sodium cholate, octylglucoside, lysophosphatidylcholine and glyceryl mono-oleate can be clearly divided, according to fluorescence data, into 2 groups. The first 4 substances, i.e., those inducing vesicle growth, behave in a very similar way and consequently only results concerning one of them, Triton X-100, will be shown here. Lysophosphatidylcholine and glyceryl mono-oleate are also very similar in their behaviour towards fluorescent probes, and only lysophosphatidylcholine data will be shown.

When the fluorescent probes ANS or NPN interact with lipids and/or detergents there is a shift in the wavelength of maximum emission towards shorter wavelengths (table 1). The marked blue shift of ANS or NPN maximum wavelength when detergents are added to the buffer solution indicates that the detergents are in the form of micelles in aqueous solution [4]. With ANS, the blue shift is bigger when the probe interacts with the lipid bilayer than with the detergent micelles. However, when increasing concentrations of Triton X-100 are added to the liposome suspension, a red shift of the maximum emission wavelength happens, of magnitude 7 nm at 0.5% Triton X-100. The final result is that λ_{max} of ANS emission is the same for a solution of pure detergent at 0.5% as for a mixture of that detergent with a liposome suspension (detergent:EYL molar ratio 16:1). The addition of similar amounts of lysophosphatidylcholine (or glyceryl mono-oleate) does not produce any red shift of the emission maximum wavelength of ANS in liposomes. There is no difference of NPN λ_{max} between pure Triton X-100 (or SDS)

Table 1
Maximum emission wavelengths of ANS and NPN in buffer, pure lipid, pure detergent and various lipid–detergent mixtures

Added detergent	Detergent:lipid molar ratio	λ_{max} in pure detergent	λ_{max} in lipid–detergent mixture
(a) ANS (λ_{max} in buffer 521 nm):			
None	–	–	475
Triton X-100 0.005%	1:6	492	474
Triton X-100 0.069%	2:1	481	475
Triton X-100 0.5%	16:1	481	481
Lysophosphatidylcholine 0.005%	1:5	484	474
Lysophosphatidylcholine 0.05%	2:1	484	474
Lysophosphatidylcholine 0.5%	18:1	483	475
(b) NPN (λ_{max} in buffer 450 nm):			
None	–	–	417
Triton X-100 0.005%	1:6	416	415
Triton X-100 0.069%	2:1	416	415
Triton X-100 0.5%	16:1	415	415
Lysophosphatidylcholine 0.005%	1:5	422	416
Lysophosphatidylcholine 0.05%	2:1	423	419
Lysophosphatidylcholine 0.5%	18:1	434	417

Measurements were reproducible to ± 1 nm. The molar ratios for phospholipid:probe molecules were of 25:1 (ANS) and 167:1 (NPN)

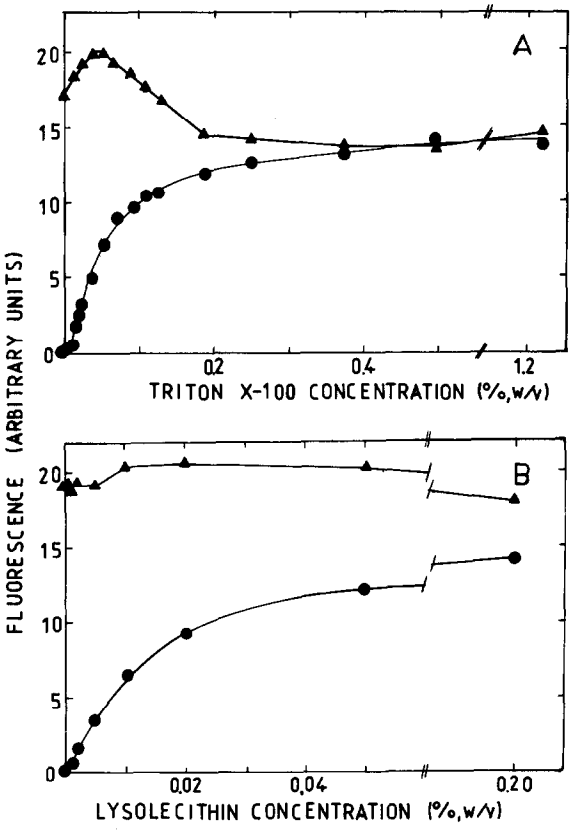

Fig.1. Effect of surfactants on the fluorescence of ANS (20 μM) in buffer (●) and in the presence of phosphatidylcholine liposomes (▲): (A) Triton X-100; (B) lysophosphatidylcholine. The lipid:probe molar ratio is 25:1.

and Triton X-100 phosphatidylcholine mixtures in the range of concentrations studied. In lysophosphatidylcholine–phosphatidylcholine mixtures, the behaviour of NPN is similar to that of ANS: λ_{max} is blue-shifted in lipid with respect to detergent, and the addition of detergent to liposome suspensions does not produce any red shift.

When ANS interacts with increasing concentrations of Triton X-100 in buffer (fig.1A) the fluorescence intensity is gradually increased until it reaches a plateau at ~0.25% Triton X-100. In the presence of liposomes and detergent, two cases must be considered: at low Triton X-100 concentrations the ANS fluorescence is greatly increased both with respect to ANS in buffer and in detergent; however, above a certain point, the fluorescence intensity of ANS actually decreases with increasing Triton X-100 concentrations. At 0.3% and higher detergent concentrations (detergent:EYL molar ratios >10:1) the fluorescence of ANS in the lipid–detergent mixture is the same as in the pure detergent. Turbidimetric and electron microscopic observations [1,2] show that, in this region, virtually all the lipid is in the form of mixed micelles with the detergent. Apparently, the fluorescence intensity (fig.1A) and maximum wavelength (table 1) of ANS are the same in pure or mixed micelles. The complex liposome curve in fig.1A can be interpreted as the result of 2 effects: a detergent-induced increase of the ANS fluorescence in the bilayer and a decrease of its fluorescence as the lamellar–micellar transition takes place. The effect of lysophosphatidylcholine on ANS fluorescence is essentially similar to that of Triton X-100, except that the plateau is already reached at ~0.1% detergent concentration (fig.1B). However, the effects of lysophosphatidylcholine on the fluorescence of ANS in the lipid bilayer are very modest, and rather suggest that practically no interaction takes place between the bilayer and the detergent micelles. This is in accord with the observations summarized in table 1.

The fluorescence of NPN in buffer is gradually increased and blue-shifted by increasing concentrations of Triton X-100, as was the case with ANS. When NPN interacts with phosphatidylcholine liposomes in the presence of detergents its fluorescence is enhanced in the absence or at low concentrations of detergent; then, at >0.08% Triton X-100 (detergent:EYL molar ratios >3:1) it roughly parallels the changes in fluorescence intensity of the pure detergent. As in the case of ANS, NPN fails to reveal any interaction of the liposomes with lysophosphatidylcholine or glyceryl mono-oleate.

The enhancement of the probe fluorescence intensity in the presence of Triton X-100, detected by ANS and by NPN was studied in detail. Triton X-100 at 5‰ was chosen because it effectively increases the fluorescence of ANS and NPN in the bilayer; yet the fluorescence of these probes in the pure detergent at that concentration is very low. These conditions correspond to a lipid:detergent:probe molar ratio of 25:4:1 (ANS) and 166:25:1 (NPN), respectively. In [4], with a glyceryl mono-oleate–erythrocyte membrane system, fluorescence enhancement was shown to be due to a fluidifying effect of the amphiphatic molecule. Fluorescence enhancement may result from a change in the quantum yield of the bound dye, or from an increase in the number of dye molecules bound, which could arise from the creation of new

sites or from tighter binding [5]. To estimate the limiting fluorescence enhancement when all the probe is bound to the bilayer, the liposome concentration was varied at a fixed concentration of probe, and the double-reciprocal plot extrapolated to infinite lipid concentration [6]. The quantum yield of ANS and NPN decrease on perturbation of the bilayer by 5‰ Triton X-100.

To determine the number of binding sites for the fluorescent probes and the binding constants, the data can be analysed by Scatchard plots [7] involving variation of the probe concentration at constant lipid concentration, using the values of the fluorescence of the bound probe determined from the double-reciprocal plots. This approach requires the substantial assumptions that all the binding sites are identical in dye-binding properties and that all the sites are independent [8]. The results for ANS show that the influence of 5‰ Triton X-100 (1 detergent/6 phospholipid molecules) is to increase the number of binding sites from 99–141 μmol/g, and the affinity of the bilayer for the probe, as shown by lowering the K_d from 28–17 nM. The corresponding effect on the NPN binding sites is to increase their number from 68 μmol/g in the case of the pure lipid to 160 μmol/g for the Triton-perturbed liposomes; the dissociation constant decreases from 71–15 nM. It must be recalled that, under these conditions, the detergent itself binds a negligible proportion of probe molecules. The above results are summarized in table 2, together with relevant data from other authors.

4. Discussion

The introduction of fluorescent probes into the lipid bilayer can provide information about properties of the microenvironment of the probe, such as polarity and rigidity [9], and their perturbation by added reagents [10]. The two fluorescent probes used in this study, ANS and NPN, have been chosen in order to obtain information from 2 distinct regions of the lipid bilayer, the lipid–water interface and the hydrocarbon core [11], respectively. The position of the emission maxima can provide information about the 'polarity' of the environment around the chromophore [12]. The observation of the blue-shift of ANS or NPN upon interaction with biomembranes is well documented and, although environmental constraint can also influence λ_{max}, it is usually attributed to the transfer of the probe to a less polar environment [4,13]. In this context, the blue-shift of ANS and NPN upon addition of detergent is easy to understand. ANS data (table 1) suggest that the microenvironment of the probe in the surface of the detergent micelle is, however, more polar than in the phospholipid–water interface. High Triton X-100 concentrations bring about the incorporation of most phospholipids into mixed phospholipid–detergent micelles [14], and consequently λ_{max} of ANS is the same in this system as in the case of the pure detergent. Very little perturbation of phosphatidylcholine liposomes is achieved under our conditions by lysophosphatidylcholine [2], and consequently λ_{max} of ANS in lipid

Table 2
Fluorescence characterisation of the interaction of various amphiphiles with natural and biomembranes

Membrane system	Fluorescent probe	Added amphiphile	Effect of amphiphile	Decrease of quantum yield[a]	Increase of binding sites[b]	Source
Sonicated EYL liposomes	ANS	Triton X-100[c]	Vesicle growth	Yes	Yes	These data
Sonicated EYL liposomes	NPN	Triton X-100[c]	Vesicle growth	Yes	Yes	These data
Sonicated EYL liposomes	ANS	Glyceryl mono-oleate[d]	None	No	No	These data
Sonicated EYL liposomes	NPN	Glyceryl mono-oleate[d]	None	No	No	These data
Erythrocyte ghosts	ANS	Glyceryl mono-oleate[d]	Fusion	Yes	Yes	[4]
Erythrocyte ghosts	NPN	Glyceryl mono-oleate[d]	Fusion	Yes	Yes	[4]

[a] Calculated from double-reciprocal plots fluorescence *vs* liposome concentration
[b] Calculated from Scatchard plots [4,7]
[c] Or SDS, sodium cholate or octylglucoside; [d] Or lysophosphatidylcholine

does not change upon addition of lysophosphatidylcholine. In addition, emission maxima values indicate that, in the presence of both phospholipid bilayers and lysophosphatidylcholine micelles the probe partitions preferentially into the bilayers. The 'deep' probe NPN does not allow us to distinguish between the microenvironments of the hydrocarbon cores of Triton X-100 micelles and those of the phosphatidylcholine bilayers. For the interaction of NPN with lysophosphatidylcholine micelles the same considerations as in the case of ANS apply.

Triton X-100 (as well as SDS, sodium cholate or octylglucoside) at low concentrations, decreases the quantum yield of ANS and NPN (table 2). This can be attributed to an increased ability of the solvent molecules to reorient around the chromophore dipole within the excited state lifetime, that is to say, to a decrease in the environmental constraint [9]. This is confirmed by the fact that, upon addition of Triton X-100, the affinity of the bilayer for the probe is increased, fluorescence intensity is enhanced, and more probe molecules are bound. Higher concentrations of detergent induce the phospholipid lamellar–micellar phase transition, and the fluidising effect of Triton X-100 is marked by the decrease in fluorescence intensity that takes place when the fluorescent probes in the bilayer are transferred to a more polar environment, i.e., the micelles. None of these effects are produced when lysophosphatidylcholine is added to the liposome suspension.

Our results suggest that the fluidising effect of lysophosphatidylcholine or glyceryl mono-oleate, that do not induce any increase in size of sonicated phosphatidylcholine vesicles, is much smaller than the effect produced by other soluble amphiphiles that are able to promote vesicle growth. These results would agree with the different effects produced by lysolecithin and Triton X-100 on the thermotropic transition of saturated phosphatidylcholines, as revealed by calorimetric studies [15,16; A. A., unpublished). It is interesting to note that when glyceryl mono-oleate acts as a fusogenic lipid (e.g., with erythrocyte ghost membranes) it also increases the fluidity of the lipid matrix [4], thus behaving as Triton X-100 in phosphatidylcholine liposome membranes. The parallelism of the effect of:

(i) Fusogenic amphiphiles when acting on cell membranes;
(ii) Certain detergents when acting on phosphatidylcholine liposomes, as studied by spectrofluorimetric techniques (table 2);

supports the hypothesis of a common molecular mechanism for cell fusion induced by amphiphiles and phospholipid vesicle growth induced by detergents.

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