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Effect of Detergents and Fusogenic Lipids on Phospholipid Phase Transitions

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Summary. The interactions of a series of amphiphilic molecules with a phosphatidylcholine/water system have been studied using differential scanning calorimetry. The addition of any of these molecules to a liposome preparation removes the pretransition endotherm. Moreover, the presence of increasing concentrations of Triton X-100 in the lipid bilayer produces a gradual decrease of the phospholipid transition temperature and enthalpy. Similar effects are produced by other detergents such as sodium dodecylsulfate, sodium cholate or octylglucoside. On the other hand, lysolecithin increases the transition temperature and leaves unaffected or increases the transition enthalpy, up to a certain lysolecithin/phosphatidylcholine ratio. These results are discussed in relation to the ability of some surfactants to induce an increase in the size of sonicated liposomes, and also in relation to previous fluorescence studies on the interaction of these detergents with phospholipid bilayers. Only those surfactants that decrease the bilayer transition enthalpy and increase the binding of fluorescent probes to the membrane hydrophobic matrix are able to induce liposome increase in size.

Key words detergent · membrane fusion · liposomes · differential scanning calorimetry

Introduction

Studies using differential thermal analysis, as well as differential scanning calorimetry have shown the existence of thermotropic phase transitions in simple phospholipid/water systems (Chapman, Williams & Ladbrooke, 1967). In the case of DPPC[1]/water systems which form an array of lipid bilayers, it has been shown that the main endothermic phase transition of the lipid can be shifted to higher or lower temperatures and sometimes removed depending on interactions of the lipid with other components, such as polypeptides, proteins (Chapman, Urbina & Keough, 1974; Papahadjopoulos et al., 1976; Gómez-Fernández et al., 1980), cholesterol (Ladbrooke, Williams & Chapman, 1968; Mabrey, Mateo & Sturtevant, 1978), alcohols, fatty acids and various sorts of drugs (Eliasz, Chapman & Ewing, 1976; Jain & Wu, 1977; Mountcastle, Biltonen & Halsey, 1978). Calorimetric studies of lipid mixtures have also been performed (Papahadjopoulos et al., 1973, 1977; Blume, Arnold & Weltzien, 1976; Kantor et al., 1977), especially in relation to vesicle fusion.

Previous studies from this laboratory have shown (Alonso et al., 1981, 1982*a*) that some detergents, such as Triton X-100, SDS, sodium cholate or octylglucoside, induce an increase in size of sonicated liposome suspensions, that could be associated to liposome fusion. Other amphipathic molecules, e.g. lysolecithin, that are known as cell fusogens (Howell & Lucy, 1969) are not active in promoting liposome fusion. We have also shown, by means of spectrofluorimetric techniques (Alonso et al., 1982*b*), that the detergents inducing an increase in vesicle size interact with the lipid bilayer much more strongly than those that do not show that effect. The present study deals with the interaction of fusogenic and nonfusogenic amphiphathic molecules with DPPC bilayers, as revealed by DSC. We show that, when the detergents are added externally to an already-made multilayered phospholipid suspension, lysolecithin interacts in a very different way from the molecules inducing liposome "growth" in size.

Materials and Methods

DPPC was obtained from Fluka, and used without further purification. Lysolecithin was from Sigma. The characteristics of the detergents have been described elsewhere (Alonso et al., 1982*a*).

Samples for differential scanning calorimetry were prepared as follows. DPPC (4 mg) were made up in excess double-distilled water (10 μl) and mixed on a bench vibrator above

[1] *Abbreviations:* DPPC, dipalmitoylphosphatidylcholine; SDS, sodium dodecylsulfate; T_c, onset temperature of the main thermotropic transition of a hydrated phospholipid; T_m, midpoint transition temperature of a hydrated phospholipid; differential scanning colorimetry.

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the lipid transition temperature (T_c). To this were added another 10 µl of double-distilled water, or of the adequate detergent solutions in order to obtain the required detergent/phospholipid ratios. The ternary system so obtained was again carefully mixed on the vibrator above T_c and allowed to equilibrate for at least 30 min at the same temperature. To obtain reproducible results careful mixing was found to be important. Ten µl of the mixture were then transferred to Perkin-Elmer aluminium "volatile" sample pans.

Calorimetric studies were carried out on a Perkin-Elmer DSC-1B differential scanning calorimeter operating in the low-temperature mode, with liquid N_2 as the coolant with a heating or cooling rate of 4° C/min. Peak areas were measured by weighing paper cut-outs of the peaks. The instrument was calibrated with cyclohexane and indium standards. The phospholipid contents of the pans were determined as lipid phosphorus according to Bartlett (1959). The appropriate blanks for the phosphorus assay were run with empty pans and the various detergents.

At least two runs were performed on each sample, neglecting the data from the first run, and at least two different samples were prepared for each experimental point. The transition temperature was considered to be the temperature corresponding to the maximum in the calorimetric curve (T_m) since accurate measurements of the onset temperature T_c of broad transitions were found to be difficult (Eliasz et al., 1976). The heats of transition were found to be reproducible to better than 10% except where transitions were very broad; the widths of transition at half height were reproducible to better than 10%, and the transition temperatures were reproducible to better than 0.5° C.

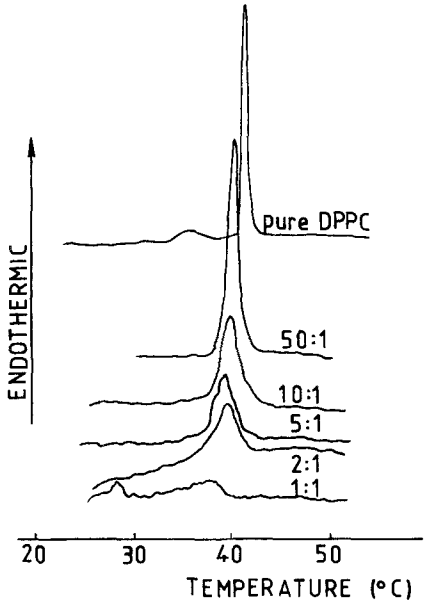

Fig. 1. Calorimetric heating curves for pure DPPC and various mixtures of DPPC and Triton X-100 in excess water. Heating rate 4° C/min. The phospholipid/detergent molar ratios are indicated on the corresponding curves

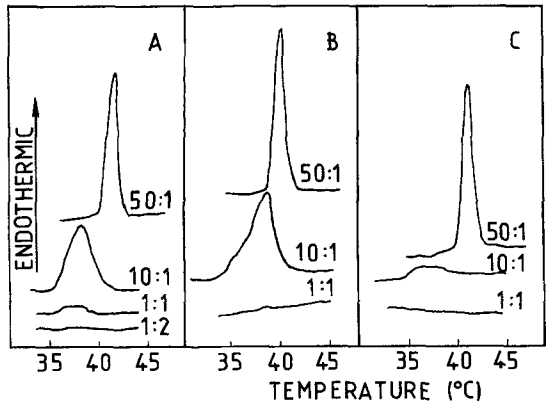

Fig. 2. Calorimetric heating curves for various mixtures of DPPC with (*A*) SDS, (*B*) octylglucoside or (*C*) sodium cholate. Heating rate 4° C/min. The phospholipid/detergent molar ratios are indicated on the corresponding curves

Results

Heating curves for detergent/DPPC mixtures, over a range of relative concentrations, all in excess water, are shown in Figs. 1 and 2. The cooling curves were very approximately symmetric, although hysteretic displacement of T_m was seen. All four detergents behave in a very similar way: T_m is slightly depressed, the main endotherm is widened and shortened as more detergent is added to the bilayer, and eventually disappears. Figure 3 shows heating and cooling curves for lysolecithin/DPPC mixtures. Lysolecithin is also a surface-active molecule, but its effects on the thermotropic transition of the DPPC bilayer are different from those of the detergents considered above. The heating and cooling curves are very different in shape, the latter being often asymmetrical. In addition, lysolecithin seems to have little effect on the size and shape of the main heating endotherm up to a 1:2 detergent/phospholipid ratio, above which the endotherm is suddenly eliminated.

These differences are more clearly seen in Fig. 4, where the gel-to-liquid crystalline transition enthalpies are plotted as a function of the detergent molar ratio for Triton X-100 and lysolecithin. The addition of increasing amounts of Triton X-100 produces a gradual decrease in the heats of transition, suggesting that a smaller number of DPPC molecules are participating in the cooperative thermotropic transition. On the contrary, lysolecithin seems first to increase the transition enthalpy change, but later this is abruptly and completely eliminated. The pretransition endotherm disappears in all cases as soon as some foreign substance is introduced in the bilayer. The transition temperature and the widths of transition at half height are shown in Figs. 5 and 6. It is clear that Triton X-100 and lysolecithin behave in a different way again. Whereas Triton X-100 produces a decrease

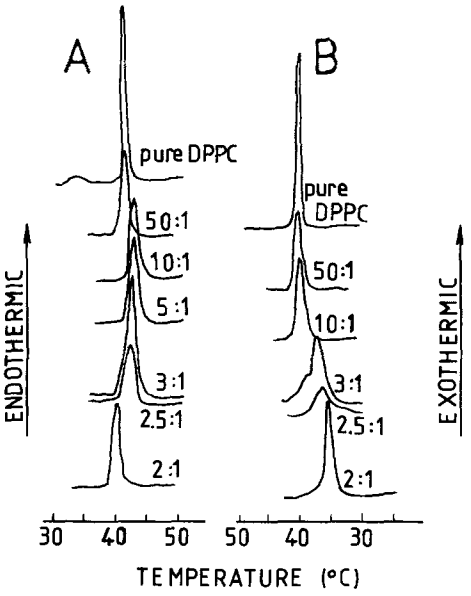

Fig. 3. Heating and cooling calorimetric curves for various mixtures of DPPC and lysolecithin. Heating rate 4° C/min. The phospholipid/detergent molar ratios are indicated on the corresponding curves. (*A*) heating curves; (*B*) cooling curves

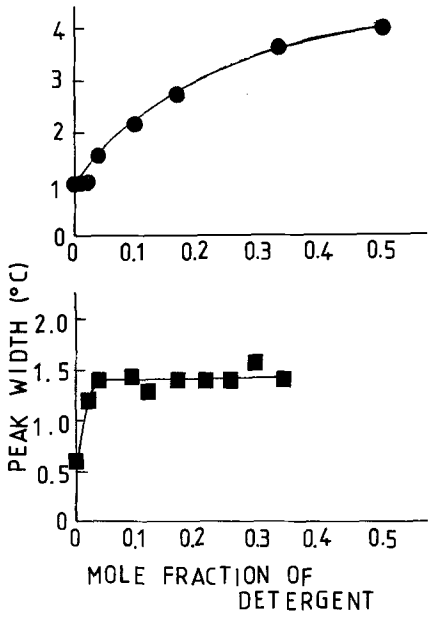

Fig. 5. Effect of varying radios of detergent to phospholipid on the width of the transition at half-height of the heating curves of the DPPC/detergent/water systems. (●) Triton X-100; (■) lysolecithin

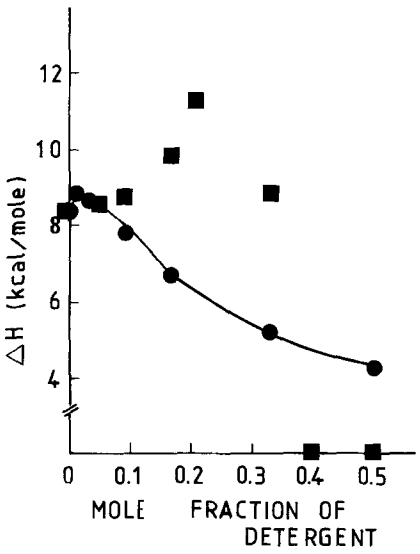

Fig. 4. Gel-to-liquid crystalline main transition enthalpies for detergent/DPPC mixtures. (●) Triton X-100; (■) lysolecithin

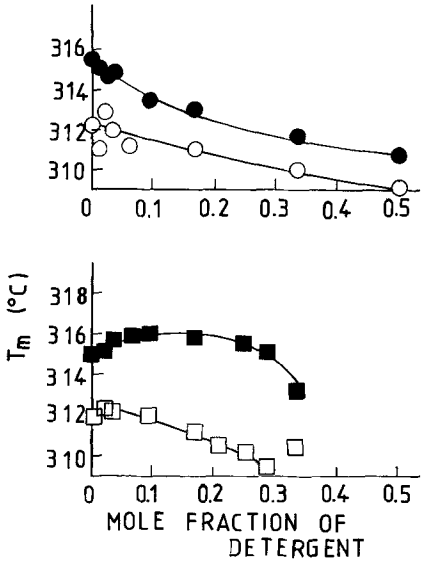

Fig. 6. Transition temperatures T_m for DPPC/detergent/water systems plotted against the molar fraction of detergent over detergent + phospholipid. (○ ●) Triton X-100; (□ ■) lysolecithin. Filled symbols: heating curves (*solidus* line); open symbols: cooling curves (*fluidus* line)

in T_m both in the heating and cooling experiments, T_m is virtually unaltered, or perhaps slightly increased, in the heating runs of the lysolecithin-containing mixtures, while it is decreased in the corresponding cooling runs (Fig. 6). Also Triton X-100 increases gradually the width of the heating (and cooling) main endotherm (Fig. 5). The increase in width produced by lysolecithin in the heating endotherm is independent of the detergent/phospholipid ratio. The widths of the cooling curves in

the lysolecithin system are very irregular, sometimes larger and sometimes smaller than those of the corresponding heating curves.

The behavior of SDS, sodium cholate or octylglucoside with respect to transition temperatures, enthalpies and widths was essentially similar to that of Triton X-100 (*data not shown*).

Discussion

The present consensus on biomembrane structure is that a lipid bilayer is the basic matrix on which membranes are built. The lipid may be in a more or less fluid condition. Variations on the fluidity of the lipid bilayer can bring about different physiological processes, one of which is membrane fusion (Kennedy & Rice-Evans, 1976; Alonso et al., 1982*a, b*).

The DPPC/water system forms spontaneously a convenient array of lipid bilayers which has been studied by many researchers (*see* Chapman, 1975, for a review). The lipid undergoes two endothermic transitions which occur at 34.5° C (the pretransition) and at 41.5° C (the main transition). At the main transition the enthalpy is 8.2 to 9.6 kcal/mole (Chapman, 1975; Mabrey et al., 1978). This main transition has been associated with the gel-to-liquid crystalline "melting" of the lipid hydrocarbon chains. Our present study is concerned with the interaction of a range of "foreign" molecules with these lipid bilayer systems and their effect on the main phase transition.

The "foreign" molecules under consideration are all surface active agents, that can be divided into two groups: the first one consists solely of lysolecithin, a lipid showing fusogenic properties on cell membrane systems, but not on liposomes (Howell & Lucy, 1969; Alonso et al., 1982*a*) and the second one consists of the surfactants Triton X-100, SDS, sodium cholate and octylglucoside, that can induce "fusion" (or increase in size) of sonicated DPPC vesicles above T_c (Alonso et al., 1982*a*). As this study concerns the detergents as potential fusogenic agents, the lipid-detergent mixtures have been prepared so that the detergent was externally added to the already-made liposome preparation, just as the fusogenic lipid is added to the cell suspension. It could be argued that the external addition of fusogen does not guarantee complete equilibration along the internal lamellae. However, turbidimetric, fluorescence and permeability experiments (A. Alonso, *to be published*) suggest that detergent equilibration is complete within seconds. In our case, equilibration was allowed to take place for at least 30 min at a temperature above T_c (*see* Materials and Methods).

Triton X-100 and the other detergents of this group act by gradually broadening the transition width and lowering its enthalpy as more and more foreign molecules interact with the bilayer. The width of the peak is considered to reflect the cooperativity of the transition (Chapman, 1975). A narrow peak indicates a highly cooperative transition; a broadening of the peak suggests a reduction in the cooperativity. If, as in the case of Triton X-100, the reduction in cooperativity is accompanied by a reduction in transition enthalpy, it is normally concluded that the additive penetrates the bilayer and withdraws a proportion of the lipid from participation in the transition. This behavior is typical of molecules such as cholesterol or intrinsic proteins, that have been shown to interact strongly with the paraffin lipid chains, being deeply embedded in the bilayer (Chapman, 1975). Triton X-100 in particular shows the same behavior found by Eliasz et al. (1976) in compounds with a hydrophobic chain of 8 to 10 carbon atoms; the hydrophobic chain of Triton X-100 contains a *t*-octyl radical; this is in accord with the assumption that only the hydrophobic part of the detergent is embedded in the bilayer. A major difference, however, between Triton X-100 or other detergents and intrinsic proteins is that, in the latter case, the reduction in transition enthalpy is due to lateral segregation of the proteins (Gómez-Fernández et al., 1980; Chapman, Gómez-Fernández & Goñi, 1982), whereas in the case of surfactants the phospholipids are partially or totally secluded from the bulk lipid phase when they become integrated in detergent-phospholipid mixed micelles inside the bilayer (Helenius & Simons, 1975). The broadening of the phase transition and decreasing of the corresponding ΔH are not due to bilayer solubilization, because, at least in the case of Triton X-100, this only happens at detergent/lipid molar ratios higher than 1:1 (Alonso et al., 1981).

On the other hand, lysolecithin slightly increases the transition temperature T_m and it does not decrease gradually the transition heat. These are characteristics shown by some extrinsic proteins and polypeptides, like ribonuclease or polylysine, that are assumed to bind the bilayer surface without interacting with its hydrophobic core (Papahadjopoulos et al., 1976). We suggest, on the basis of calorimetric data, that lysolecithin interacts with the lipid bilayer in a different way than Triton X-100, possibly via weak electrostatic bonding to the headgroup region of the phospholipids.

Studies concerning the thermotropic behavior of DPPC in the presence of lysolecithin have been published by other authors (Blume et al., 1976).

In those cases lysolecithin was either mixed in chloroform solution to DPPC prior to the preparation of liposomes, or was added *a posteriori* as in our case. The results are similar in all cases, namely, that lysolecithin, at molar ratios up to about 1:1, hardly affects the temperature or width of DPPC transition. Our results are also in accord with those of Jain and Wu (1977) on the effect of various detergents on the main thermotropic transition of DPPC.

Our observations are interesting in view of the "fusogenic" effects of Triton X-100 on DPPC bilayer, i.e., its ability to promote an increase in liposome size; no similar effect of lysolecithin has been observed (Alonso et al., 1982*a*). These experiments were carried out on sonicated liposomes; the thermotropic behavior of these vesicles is very complex, and so the present study has been carried out with multi-shelled liposomes. Because of this fact, the results contained in the present paper should be applied only with caution to the case of sonicated liposomes. Nevertheless, further work from our laboratory has shown, by means of spectrofluorimetric techniques (Alonso et al., 1982*b*), that surfactants interact in much the same way with the hydrophobic core of both, sonicated and nonsonicated liposomes. These observations support the application of the calorimetric results to the case of single-shelled vesicles. Our fluorescence and calorimetric studies indicate that, with regard to the phenomenon of detergent-induced increase in liposome size, each detergent interacts in its own peculiar way with the lipid bilayer. Only detergents that (i) from the point of view of calorimetry, decrease the transition temperature and enthalpy of saturated phospholipid bilayers, while (ii) in fluorescence studies, decrease the quantum yield and increase the binding sites of extrinsic hydrophobic fluorescent probes, can, in the case of sonicated vesicles, give rise to larger liposomes. The connection of these observations with the physiological phenomenon of membrane fusion remains, however, to be established.

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