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Fusion of vesicles with the air–water interface: the influence of polar head group, salt concentration, and vesicle size

M. Gugliotti *, H. Chaimovich, M.J. Politi

Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, Av. Prof. Lineu Prestes 748, 05508-940 São Paulo, Brazil

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Abstract

Fusion of vesicles with the air–water interface and consequent monolayer formation has been studied as a function of temperature. Unilamellar vesicles of DMPC, DPPC, and DODAX ($X = Cl^-$, Br^-) were injected into a subphase containing NaCl, and the surface pressure (tension) was recorded on a Langmuir Balance (Tensiometer) using the Wilhelmy plate (Ring) method. For the zwitterionic vesicles, plots of the initial surface pressure increase rate (surface tension decrease rate) as a function of temperature show a peak at the phase transition temperature (T_m) of the vesicles, whereas for ionic ones they show a sharp rise. At high concentrations of NaCl, ionic DODA(Cl) vesicles seem to behave like zwitterionic ones, and the rate of fusion is higher at the T_m. The influence of size was studied comparing large DODA(Cl) vesicles with small sonicated ones, and no significant changes were found regarding the rate of fusion with the air–water interface.

Keywords: Vesicles; Phase transition temperature; Surface pressure; Fusion; Interface

1. Introduction

Vesicles and liposomes may disrupt upon contact with the air–water interface forming a monolayer [1–3]. As a consequence of monolayer formation, the surface pressure, π (or tension, γ) of a vesicle dispersion changes. This phenomenon has been detected in dispersions of vesicles and liposomes made of different types of lipids, natural and synthetic ones [4,5], and kinetic studies [6–8] have been developed to characterize the process. Fusion (or spreading) of vesicles at the surface of solutions has been used as an alternative to form monolayers with attached proteins [9,10]. Most of the works are focused on the influence of temperature on the rate of vesicles fusion with the air–water interface, which has various implications of biological and pharmacological interest, since the transformation of a closed bilayer into a monolayer is an alternative to study the problem of the lung surfactant [11,12].

Fusion of vesicles with the air–water interface is a spontaneous process, and the large influence of the phase transition temperature of vesicles (T_m) [13] on the rate of fusion has been noticed. Fusion becomes much faster as the temperature of the system approaches the T_m [13–15]. The correlation between temperature and rate of fusion leads to the possibility of studying bilayers properties like permeability and interbilayer fusion using this phenomenon, since these properties depend also strongly on the temper-

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* Corresponding author. Fax: +55-11-815-5579; E-mail: bat@quim.iq.usp.br

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ature and are related to structural changes on the membranes. Recently, we showed that is possible to determine the T_m of vesicles using this methodology [16,17]. The aim of the present work is to study the differences between ionic and zwitterionic vesicles regarding the rate of fusion with the air–water interface below and above the T_m. From the results is also possible to estimate de T_m of the vesicles. DODA(Cl) vesicles were chosen to verify the influence of salt concentration and size.

2. Materials and methods

2.1. Materials

Dioctadecyldimethylammonium chloride and bromide (DODA(Cl)/DODA(Br)) were purchased from Kodak, and recrystallized using methanol/acetone 5:95 (v/v). NaCl (Merck) was of analytical grade. Dimyristoyl/dipalmytoylphosphatidylcholine (DMPC/DPPC) were purchased from Sigma. DPPC and DMPC were analyzed by thin-layer chromatography and showed just one spot. The water used in all experiments was doubly distilled and filtered using a Milli-Q (Millipore) apparatus.

2.2. Methods

2.2.1. Vesicle preparation and properties

The concentrations of all vesicle dispersions used are expressed in terms of the lipid monomer concentrations.

Different volumes of small unilamellar vesicles dispersions were prepared by sonicating (15 min) lipid suspensions (4 and 10 mM) at about 10°C above the T_m of the vesicles using the titanium tip sonicator Braunsonic 1510 (B. Braun). The NaCl solutions for sonication were of the same concentration of those used for the surface pressure (tension) assays, in order to ensure an isosmotic medium. After sonication, the vesicle dispersions were centrifuged ($10\,000 \times g$ for 20 min) in order to remove the titanium particles. Vesicle size was determined by dynamic light scattering (He–Ne laser, 60 mW, EMI photomultiplier, Brookhaven BI90 autocorrelator). Vesicle hydrodynamic diameters were 360 ± 50, 400 ± 50, 450 ± 50, and 570 ± 50 Å for DODA(Cl), DODA(Br), DMPC, and DPPC, respectively. Large unilamellar DODA(Cl) vesicles were prepared by the injection method [18] yielding vesicles with a diameter of 3800 ± 50 Å. The NaCl concentration for the light-scattering assays was 1 mM for all types of vesicles.

2.2.2. Surface pressure (π) and surface tension (γ) measurements

The Teflon trough consisted of a hollow circular compartment (diameter 6 cm) connected to a water bath circulator system. The temperature was measured inside the trough using a Teflon-coated thermometer (0.5°C accuracy). Vesicle aliquots were injected into the subphase using a glass pipette, followed by magnetic stirring using a Teflon stir bar. π was measured by the Wilhelmy plate method [19] on a Langmuir balance (KSV Instruments, model KSV 5000), placed in a clean room. The same procedure was carried out using the Ring method [20], and γ was measured on a Du Nouy tensiometer (Fisher Scientific Tensiomat, Model 21, Fisher Scientific).

2.3. Experimental procedure

The assays were carried out in four steps.

(1) An aliquot of the vesicle dispersion (1, 3, 5 ml,...) was injected into the subphase. The system was stirred very carefully during the next 10 s. Following this, the surface was cleaned by aspiration using a Teflon tube connected to a vacuum pump. After setting π to zero, the assay began at the selected temperature; (2) π was recorded continuously as a function of time; (3) after the measurement, the temperature was set to the new value; (4) when the system reached the new temperature, the surface was cleaned by aspiration and the assay restarted without further stirring. The same procedure was carried out when using the tensiometer, but in this case γ was measured every 5 min.

3. Results

3.1. Influence of polar head group

Fig. 1 shows the results obtained using the Langmuir balance for DODA(Br) and DPPC sonicated vesicles. The surface pressure π was recorded at sev-

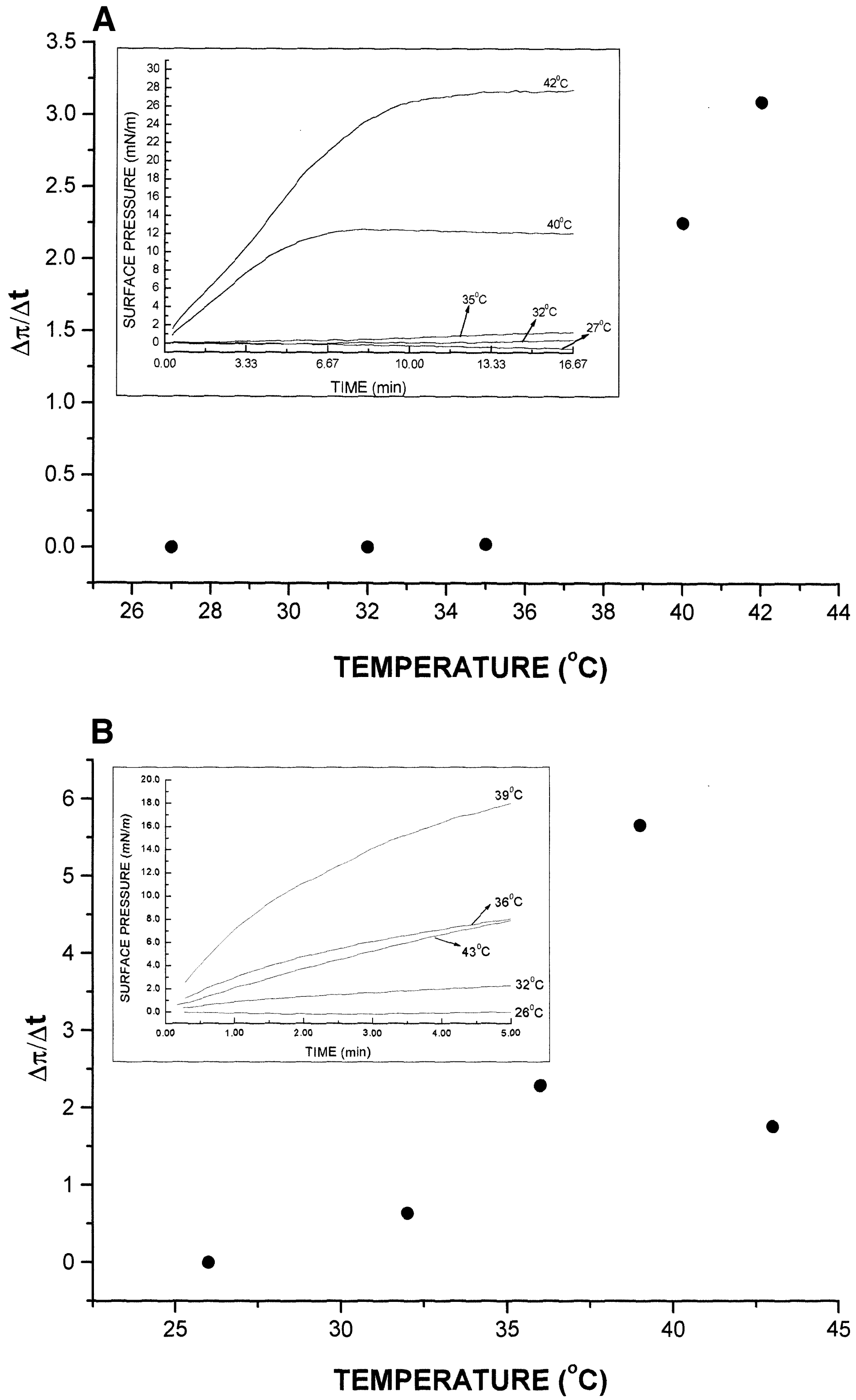

Fig. 1. (A) Δπ/Δ*t* as a function of temperature for 5 ml of 5 mM of small DODA(Br) vesicles injected into 35 ml of a 1 mM NaCl subphase. (B) Ten ml of 5 mM of small DPPC vesicles were injected into 30 ml of a 1 mM NaCl subphase. The insets show π recorded at several temperatures on a Langmuir balance.

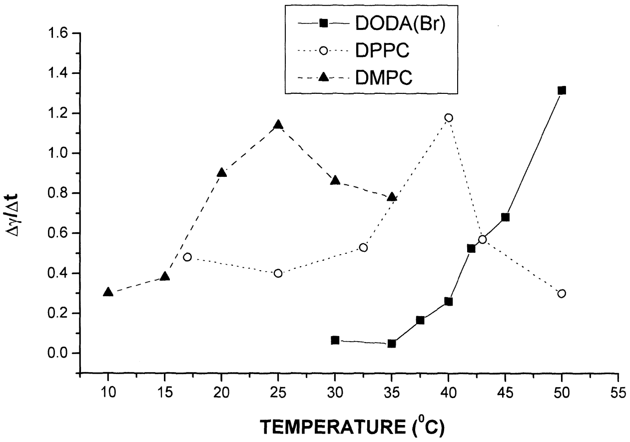

Fig. 2. Δγ/Δt as a function of temperature for 7 ml of 5 mM of small DODA(Br) vesicles (■), 5 ml of 2 mM of small DPPC vesicles (○), and 5 ml of 5 mM of small DMPC vesicles (▲). The subphases consisted of 40 ml of a 1 mM NaCl solution.

eral temperatures (Fig. 1, insets), and the tangents to the curves taking into account the first minute (Δπ/Δt, where π is the surface pressure and t is time) were calculated using KSV-LB5000 software, and plotted as a function of temperature. For DODA(Br) vesicles (Fig. 1A) it is possible to note a sharp rise in Δπ/Δt after 35°C, which corresponds to the T_m of these vesicles. A different pattern is observed for DPPC vesicles (Fig. 1B), where Δπ/Δt reaches a maximum at 39°C, and decreases thereafter. The same assay was carried out using the tensiometer for DODA(Br), DPPC, and DMPC sonicated vesicles. The surface tension decrease rate Δγ/Δt was plotted against the temperature, and the same result was found for DODA(Br) vesicles (Fig. 2). For DPPC and DMPC vesicles, Δγ/Δt showed a peak at 40°C and 25°C, respectively (Fig. 2), which are the T_m for these vesicles.

3.2. Influence of vesicle size

Fig. 3 compares the results for small and large unilamellar DODA(Cl) vesicles. From the results obtained using the Langmuir balance, Δπ/Δt was calculated and plotted against the temperature. The NaCl concentration of the subphase (1 mM) was the same for both types of vesicles. A sharp rise was found at 36°C and 37.5°C for the small and large DODA(Cl) vesicles, respectively.

3.3. Influence of salt concentration

Fig. 4 shows the results of small DODA(Cl) vesicles prepared in 10 mM and 20 mM NaCl solutions. The change on the behavior of the fusion rate was observed in the 10 mM NaCl solution, and for 20 mM of NaCl, the Δπ/Δt pattern has changed completely. For these two cases, a peak at 40°C determines the T_m, a few degrees higher than at lower concentrations of NaCl. The same result was obtained using a 50 mM NaCl solution as subphase (data not shown).

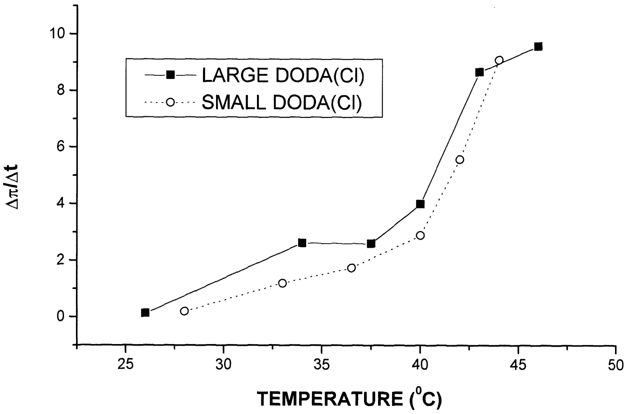

Fig. 3. Δπ/Δt as a function of temperature for 5 ml of 5 mM of small DODA(Cl) vesicles (○), and for 10 ml of 10 mM of large DODA(Cl) vesicles (■) injected into 35 ml of a 1 mM NaCl solution.

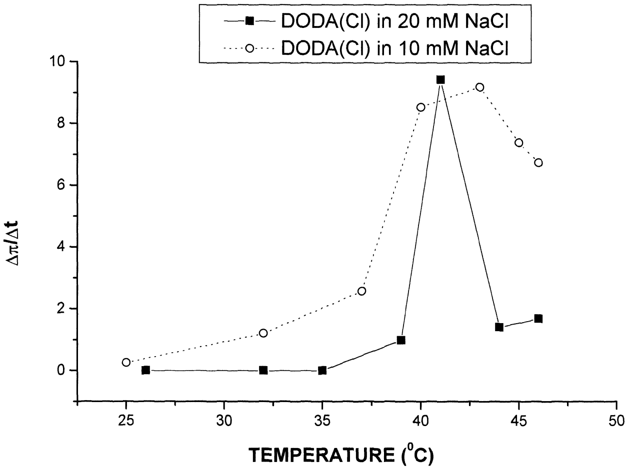

Fig. 4. Δπ/Δt as a function of temperature for 10 ml of 5 mM of small DODA(Cl) vesicles injected into 30 ml of NaCl, 10 mM (○) and 20 mM (■).

Table 1
Comparison between the transition phase temperatures obtained using the tensiometer and the Langmuir balance with the literature values for T_m of sonicated vesicles

Vesicles	Tensiometer (°C)	Langmuir balance (°C)	Literature value (°C)	Refs.
DODA(Cl)	–	36	37.5	[17,18,28]
DMPC	25	–	22–29	[29][b]
DPPC	40	39	41	[30,31]
DODA(Br)	35	35	36–40[a]	[31–33]

[a] Different values between 36°C and 40°C are found for DODA(Br) in the literature.
[b] Ref. [29] is a database containing 702 records on the T_m of DMPC vesicles.
The most common temperature found is 24°C.

4. Discussion

The biggest advantage of this method is that the rate of fusion does not depend on the concentration of the vesicles when high-concentration samples are used [12,14]. According to the results obtained by Pattus and Schindler [2,3], 75% of the vesicles remain like layers directly beneath the water surface in equilibrium with those in the bulk. This equilibrium is governed by the diffusion of the vesicles to the bulk, which changes when low-concentration samples are used. In addition, the equilibrium between the vesicles is affected during the assay by aspirating the surface, because part of the vesicle population may be lost, decreasing the concentration. Some authors have reported changes on $\Delta\pi/\Delta t$ when very low concentrations of vesicles are used [21,22]. Besides, even where using high concentrations of vesicles (5, 10 mM) to avoid this, fluctuations may occur because of the raising and lowering of the ring, when using the tensiometer, due to the change in the equilibrium between the vesicles near the interface and those in the bulk. Thus, for a simple verification, all assays were repeated at least two times for all types of vesicles, and with several different total vesicle concentrations, no change in the results was found. A more accurate determination of the T_m by this methodology is possible using lower concentrations of vesicle dispersions. In this case, π or γ are recorded continuously as a function of temperature, without any interference at the surface (aspiration) during the assay [16,17]. However, in this approach is not possible to detect differences between ionic and zwitterionic vesicles. The influence of the polar head group is clearly observed in Figs. 1 and 2. It is interesting to note that, for zwitterionic vesicles, plots of $\Delta\pi/\Delta t$ (or $\Delta\gamma/\Delta t$) show similar results to those obtained from permeability studies [23,24], where the maximum in permeability occurs when both the gel and liquid–crystalline phases coexist. The results presented here indicate that the zwitterionic vesicles are relatively stable below and even above the T_m, and at this temperature they fuse with the interface easily. On the other hand, the ionic vesicles showed a continuous increase in the rate of fusion above the T_m. In fact, an increase in permeability of DODA(Cl) vesicles at temperatures above the T_m has been reported [25].

It is known that DODA(X) ($X = Cl^-$, Br^-) vesicles are not stable in the presence of high concentrations of salt [26]. Vesicle dispersions prepared with 10 mM of NaCl did not show high turbidity, but the samples prepared with 20 and 50 mM of NaCl were very turbid. However, the addition of salt from 10 up to 50 mM showed the same change on the fusion rate of DODA(Cl) vesicles, that is, a peak instead a sharp rise at the T_m, with a slight increase on its value. This effect is assigned to the lowering of the degree of ionization of the vesicles as a function of the increase in salt concentration in the subphase. As the ionic strength increases, the repulsion between the charged head groups decreases, allowing the lipids to stay closer to each other [27]. This represents a gain in stability, and it is seen as an increase on the T_m.

No significant changes were found between the rate of fusion with the interface for large and small DODA(Cl) vesicles, although different results are found in the literature [3,6]. It is possible to observe that the sharp rise on $\Delta\pi/\Delta t$ occurs at 36°C and 37.5°C for the small and large DODA(Cl) vesicles, respectively, which corresponds to the T_m region for these vesicles.

Table 1 summarizes the results for T_m determination obtained using the tensiometer and the Langmuir balance for each type of vesicle, and the literature values for T_m are also listed for comparison. The T_m values found by analyzing the rate of fusion are in good agreement with those obtained by other techniques (see references in Table 1).

5. Conclusions

The T_m values obtained using the tensiometer and the Langmuir balance are nearly the same, and the results are in good agreement with those obtained by other methods. A difference in the behavior between zwitterionic and ionic vesicles at low salt concentration is observed. The results show that the ionic vesicles continue to fuse with the air–water interface above their T_m, while for the zwitterionic ones the maximum rate of fusion occurs at this temperature. The addition of salt to the ionic vesicles changed their behavior into that of zwitterionic ones. Although DODAX (Cl^-, Br^-) vesicles do not present good stability in the presence of salt, it was possible to detect the change on the rate of fusion with the air–water interface at the T_m, and a change on its value due to high salt concentrations of NaCl. No significant changes on the T_m values were found between small and large DODA(Cl) vesicles, since the difference of 1.5°C is within experimental error. These results strongly suggest that the mechanism of fusion with the interface for DODA(Cl) vesicles does not depend on the curvature radii of these bilayers.

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