EFFECT OF DESOXYLYSOLECITHINS ON DIMYRISTOYLLECITHIN VESICLES

Influence of the lipid phase transition

Karl HARLOS, Winchil L. C. VAZ and Stephan KOVATCHEV
Max-Planck-Institut für Biophysikalische Chemie, D-3400 Göttingen-Nikolausberg, FRG

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1. Introduction

One of the important characteristics of phospholipid–water lamellar phases is the ordered–disordered phase transition [1]. It is not as yet clearly understood what role this phase transition may play in the dynamic aspects of biomembrane behaviour. Among other interesting aspects, the lipid phase transition has been reported to have a significant effect upon the 'mixing' of phospholipid bilayer vesicles [2], permeability of the bilayer [3–5] and segregation of components in the plane of the bilayer [6].

Small amounts of lysophospholipids are known to be present in cells [7] and some vital cellular processes such as cell-fusion [8], the immune response [9] and enzyme activities [10] may be influenced by lysophospholipids. The effects of lysolecithins upon lipid vesicles [11–17], as well as the lysolecithin induced hemolysis of erythrocytes [18–21] has been subjected to much study. However, to our knowledge, no detailed investigation of the kinetics of liposome lysis by lysolecithins has been reported in the literature.

In the present communication we wish to report the influence of the lipid phase transition upon the lysolecithin induced lysis of lecithin bilayer vesicles. A stopped-flow analysis has been used to study the kinetics of vesicle breakdown in the presence of sufficiently large concentrations of lysolecithins. Dimyristoyllecithin and 3-myristoylpropandiol-1-phosphorylcholine (a 2-desoxy derivate of myristoyllysolecithin) were used in the kinetic analysis.

2. Materials and methods

Dimyristoyllecithin was from Fluka AG, Buchs, Switzerland, and was purified by silica-gel chromatography before use. Lauroyl-, myristoyl- and palmitoyl-desoxyly- solecithins were synthesized according to described procedures [22] and purified by silica-gel chromatography prior to use. The lecithin and desoxylysolecithins used in this work showed only one spot on thin-layer chromatograms

For the preparation of lipid vesicle suspensions, desired amounts of the lipid were suspended in water and sonicated in an argon atmosphere at 15–20°C in a bath-type sonifier until the suspension became clear (usually 2 h). The suspensions were then kept at 40°C for 30 min to completely anneal any lattice defects in the vesicles [23].

Vesicle suspensions and mixed dispersions were negatively stained at room temperature with 2% uranyl acetate and examined on a Siemens 101 electron microscope operating at 80 kV with instrumental magnifications of 20 000.

Spectroscopic measurements were done on a Cary 118 Spectrophotometer and an Aminco-Bowman Ratio Spectrophotofluorometer. For kinetic studies a stopped-flow photometer, made in this institute by Mr C. R. Rabl, was used. The instrumental dead-time was 5 ms. The absorption cuvette (path-length 14 mm) and reservior syringes were thermostated at identical temperatures and sufficient thermal equilibration time (15 min) was allowed. After mixing of the lipid-vesicle sus-

pension with the lysolecithin solution the change in absorbance was monitored. The relaxation-times τ were obtained by fitting the curves to exponential functions:

$$I(t) = I(o)e^{-\frac{t}{\tau}}.$$

3. Results

The lipid vesicles used in this work have been characterized by freeze-fracture electron microscopy and NMR spectroscopy (Vaz, W.L.C., Hårlos, K. and Füldner, H.H., unpublished results). They appear to be single-shelled and have diameters of 500–1000 Å. Light scattering measurements of the lipid thermal phase transition showed a sharp drop in the light scattering intensity at the thermal phase transition temperature, T_t, without hysteresis (fig.1, top curves).

The influence of three desoxylysolecithins on the optical density at 400 nm of lipid vesicle suspensions is shown in figure 2. Equal volumes of dimyristoyllecithin suspension and desoylysolecithin solutions were mixed and the optical density was measured until no more change could be observed. In fig. 2 it can be seen that at a fixed lipid final concentration of 5×10^{-4} M, there is a drop in the optical density of the mixtures at desoxylysolecithin concentrations of from 0.1–1 mM for all three desoxylysolecithins. It

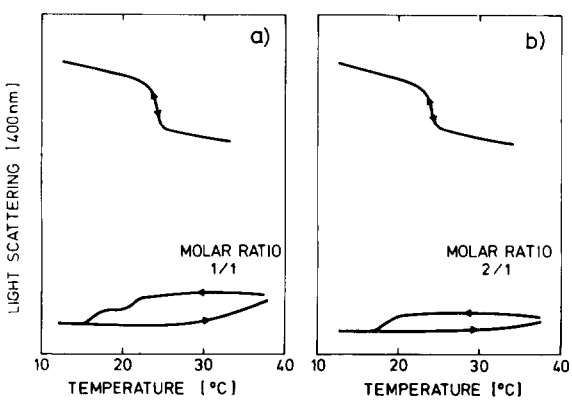

Fig.1. Light-scattering transition measurements (scan rate 30°C/h) of the pure lecithin (DML) (top curves) and the mixed lysolecithin (C_{14}dLL)/DML suspension with a molar ratio of (a) 1/1 and (b) 2/1. The final lipid concentration in this and in all further experiments was 5×10^{-4} M.

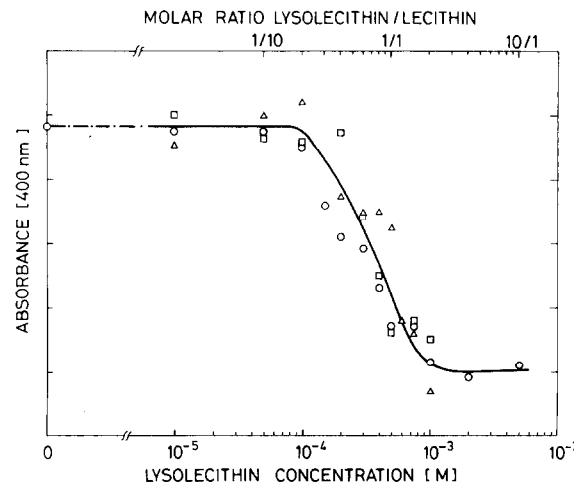

Fig.2. Absorbance (400 nm) of dLL/DML suspensions at 25°C for three different lysolecithins: (△) C_{12}dLL, (○) C_{14}dLL, (□) C_{16}dLL. The decrease in absorbance at the lysolecithin concentration from 0.1–1 mM is due to the breakdown of the lipid vesicles and the formation of mixed micelles.

appears therefore that independent of the desoxylysolecithin acyl-chain length, breakdown of the lipid vesicles occurs at a desoxylysolecithin/lecithin ratio of 1/1. The breakdown is generally accepted to result in the formation of mixed micelles of lipid and lysolipid [13]. Electron micrographs of negatively stained mixed dispersions (molar ratio of desoxylysolecithin to lecithin = 1/1) show that the particles appear to be similar to lecithin micelles [24] as seen in fig.3. Thermal phase transitions measurements showed an absence of the usual lipid phase transition (fig.1, bottom curves).

Kinetics of the desoxylysolecithin induced breakdown of lecithin vesicles was investigated using the myristoyl–desoxylysolecithin/dimyristoyllecithin system. The change in optical density of the mixture as a function of time was recorded on the stopped-flow photometer in order to achieve the necessary rapid mixing of the two components in the reaction mixture. Figure 4 shows the relaxation-times τ as a function of temperature for two desoxylysolecithin/lecithin ratios (final lipid concentration 5×10^{-4} M). A clear minimum of τ can be seen at the lipid T_t for desoxylysolecithin/lecithin ratios of 1/1 as well as 2/1. Below T_t the lipid vesicle breakdown is considerably slower until only

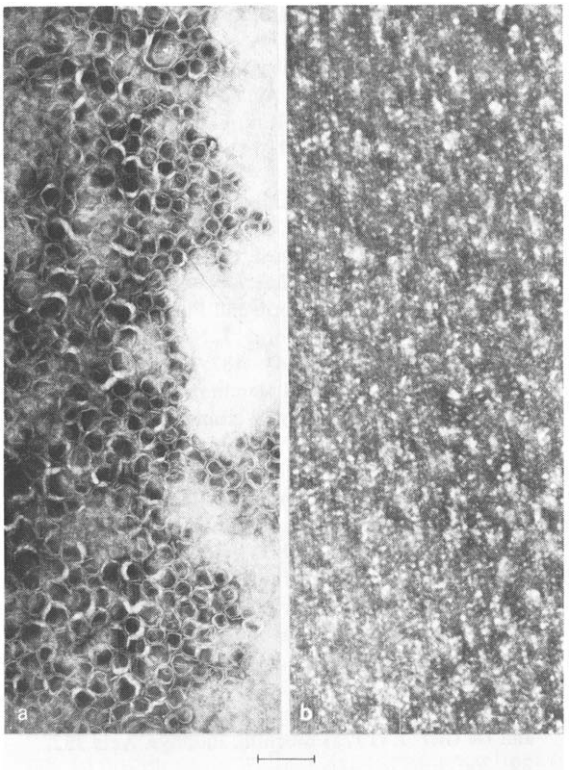

Fig.3. Negatively stained (2% uranyl acetate) electron micrographs of (a) DML vesicles and (b) mixed C_{14} dLL/DML suspensions (molar ratio 1/1). The length of the inserted bar is 2000 Å.

incomplete lysis is seen at temperatures below 20°C. Above T_t a steady increase in τ is observed. Thus the rate of breakdown of the vesicles appears to be critically dependent upon the transition temperature of the pure lipid. In fig.4 it can be seen that τ (40°C)/τ (T_t) is about 10, and τ (20°C)/τ (T_t) is about 10^3. Also, it is interesting that the value of τ at T_t does not appear to be ratio-dependent.

4. Discussion

Four steps have been proposed to be involved in the lysis of lipid vesicles by lysolecithins [18]

(i) Adsorption of the lysolecithin to the lipid vesicle

(ii) Penetration of the lysolecithin into the lipid bilayer

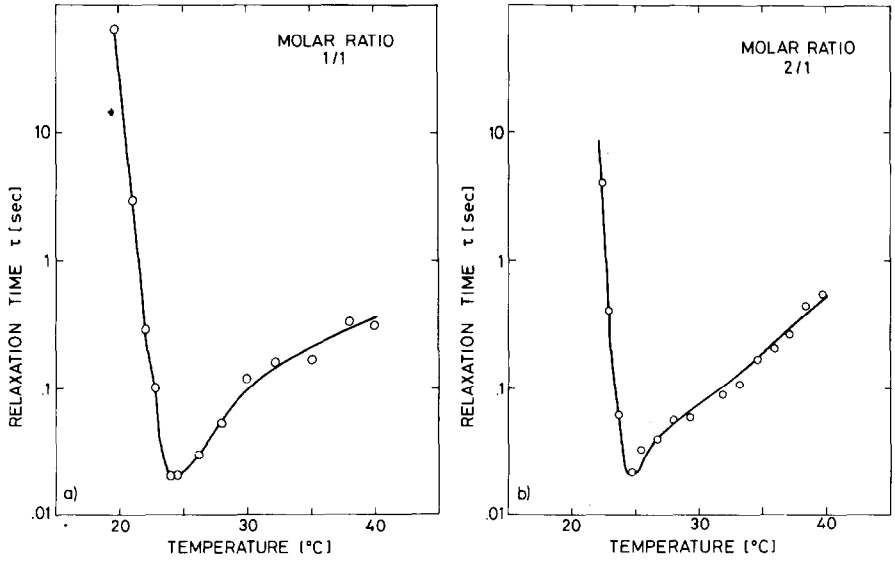

Fig.4. Relaxation times τ of the decrease in absorbance after the mixing of C_{14} dLL with DML vesicles as a function of temperature for a C_{14} dLL/DML molar ratio of (a) 1/1 and (b) 2/1.

(iii) Diffusion and distribution of the lysolecithin in the lipid bilayer

(iv) Disruption of the lipid bilayer.

It is realized that the rates seen in fig. 4 cannot be assigned in the present work to any one of these individual processes but probably reflect the rate of the rate-limiting step in the overall reaction. One could speculate, however, as to which of the above steps may be rate-limiting. Steps (i) and (ii) are probably diffusion-controlled and hence likely to be much faster than the observed rates. The critical dependence of τ on T_t of the pure lipid would suggest Step (ii) as the most likely candidate for the rate-limiting step since it is known that T_t for lysolecithin/lecithin mixtures at non-lytic lysolecithin concentrations is different from T_t for the pure lipid [16]. Earlier work has suggested that lysolecithin-induced liposome lysis requires a certain fluidity of the lipid bilayer [12]. Our results are in agreement with this finding. It would be interesting to see whether liposome lysis by detergents such as Triton X-100 [25] follows kinetic patterns similar to those reported here.

It was observed in the course of this work that there is a slow increase in the absorbance of mixed micelles of lecithin and lysolecithin at high temperatures. This increase seems to be greater at a desoxylysolecithin/lecithin ratio of 1/1 than it is at a ratio of 2/1 (see fig.1, bottom curves). Preliminary electron microscopy studies indicate that upon storing of mixed micelles at 40°C ($T >> T_t$) large particles of about 500–1 000 Å diameter are formed. It is not as yet clear whether these particles are vesicles. This subject is under further investigation.

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