Eur Biophys J (1990) 17:343–347

European Biophysics Journal

Pure and mixed lipid black foam films as models of membrane fusion

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Received May 15, 1989/Accepted in revised form September 27, 1989

Abstract. Black foam films (BFF) from water solutions of the phospholipid dilauroyl lecithin (DLL) with admixtures of palmitoyl lysolecithin (Lyso) were formed. Microscopic BFF were studied by the method of Scheludko and Exerowa. The formation probability for BFF and the BFF lifetime in a black state before film rupture were measured as functions of the film composition. At a fixed overal lipid concentration it was shown that an increased percentage of Lyso exponentially increased the lifetime of the film up to the CMC of Lyso. This stabilizing Lyso effect nicely corresponds with its stabilizing action on the waiting time for fusion of two contacting black lipid membranes (BLM), as found by Chernomordik et al. In contrast, Lyso is known to destabilize a single BLM. In this way we have found experimental proof of our earlier prediction that Lyso should have opposite effects on the lifetimes of BLM and BFF. In addition, we have shown for the first time that foam films made of lipids are a convenient model for monolayer membrane fusion studies. This model is characterized by its simplicity and experimental reliability and provides a means for quick screening of the fusogenic capacity of various amphiphilic and hydrophilic admixtures.

Key words: Dilauroyl lecithin, palmitoyl lysolecithin, black foam film stability, membrane fusion modeling

Introduction

Model studies of the stability and interactions of black lipid membranes (BLMs) are of great current interest in relation to biological membrane fusion. It is known that some lipid species may alter the extent of fusion between two BLMs of phospholipids. A recent example was provided by the investigations of the waiting time for fusion of two BLMs of phosphatidylethanolamine in the presence of increasing concentrations of lysolecithin in the bathing solution (Chernomordik et al. 1985).

In this paper we report the results of a study of the probability of formation of a black foam film, W, and the lifetime of the film, τ, i.e. the time from the appearance of the first black spot until the film breakdown, for pure DLL films and for mixed DLL/Lyso films. We have found a correlation between the increase of the lifetime of BFFs from lecithin with admixtures of lysolecithin and the increase of the fusion waiting time of two BLMs from phosphatidylethanolamine in the presence of lysolecithin.

Fusion is a type of membrane instability involving two membranes simultaneously. As a rule biomembranes are not supposed to fuse. However, in some processes such as, for example, hormone secretion and mediator release membrane fusion is a common step. It is probably mediated by some biphilic species being a product of the metabolism of arachidonic acid. Other species could act as fusion inhibitors. The present study aims to contribute to the understanding of the relationship of the so-called monolayer fusion to the shape of lipid species. Monolayer fusion is an instability of the thin water film between the two closely contacting membranes presumably taking place by formation across the narrow water gap of necks, or stalks, joining together the two opposing monolayers (Chernomordik et al. 1985) (Fig. 1 a). Basically, our idea was to model just this thin water film by a lipid stabilized black foam film shown schematically in Fig. 1 b. A foam film is essentially a thin liquid (e.g. water) film whose two interfaces are covered by two monolayers of some surfactants, in our case phospholipids. In the so-called Newton (or secondary) black state of a foam film the water gap is extremely narrow and should correspond well to the close contact state necessary for monolayer fusion. According to the nucleation theory (Kashchiev and Exerowa 1980; Exerowa and Kashchiev 1986) the rupture of black foam films involves the formation of holes (vacancies of molecules) in be-

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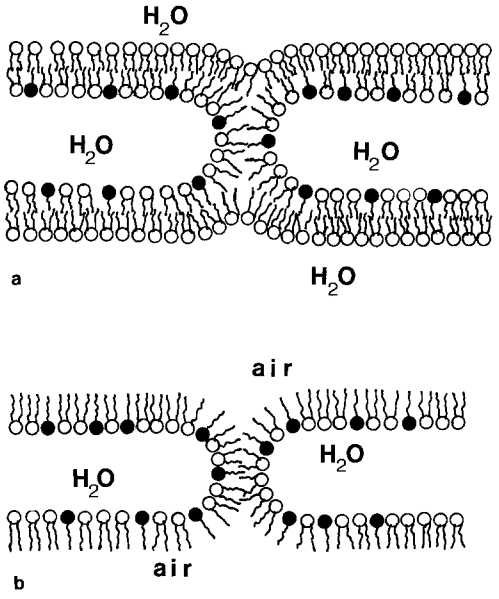

Fig. 1 a and b. Comparison of monolayer membrane fusion and black foam film rupture. Double chain lipids represent lecithin and single chain lipids represent lysolecithin. **a** neck formation at monolayer fusion. **b** neck formation at foam film rupture. In both cases lysolecithin increases the neck elastic energy (see text)

tween the two monolayers which are capable of irreversible lateral growth. Assuming a subsequent connection of the monolayers in the form of a neck (stalk), a common intermediate structure for black foam film rupture and monolayer membrane fusion could be proposed (Fig. 1).

Lysolecithin is an interesting lipid species for testing the theoretical predictions of the liquid crystal approach in membranology (Petrov and Derzhanski 1976; Petrov et al. 1979; Petrov and Bivas 1984). This approach was based on the curvature elastic energy concept (Helfrich 1973) and on the lipid shape concept (Petrov and Derzhanski 1976). Being a single hydrocarbon chain lipid, a positive steric asymmetry was ascribed to the lysolecithin shape, i.e. head group cross section larger than the chain cross section (Petrov and Derzhanski 1976). As a result of its asymmetric shape lysolecithin is expected to decrease the curvature elastic energy for positive monolayer curvature (e.g. in the edge of a membrane pore, Petrov et al. 1980; Helfrich 1981) and to increase that energy for negative curvature (e.g. in the neck between two contacting monolayers, Petrov et al. 1980; Helfrich, 1981). The nucleation theory of Exerowa et al. (1983); Exerowa and Kashchiev (1986) predicts an exponential increase of the BFF lifetime with the critical defect energy. The liquid crystal approach additionally predicts an increment to the neck peripheral energy growing linearly with the percentage of positive asymmetry lipids, so that in the low concentration limit the total energy of the critical defect will also rise linearly with the Lyso concentration. Thus, if a common intermediate for fusion and rupture, as shown in Fig. 1, really exists a similarity of the stabilizing lysolecithin effect on both systems is to be expected.

Materials and methods

DL-β, γ-Dilauroyl-α-lecithin (DLL) and DL-γ-Palmitoyl-α-lysolecithin (Lyso) were obtained from Fluka and were used without further purification. According to the Fluka catalogue, DLL was puriss grade, ca. 99% purity checked by gas chromatography, after transesterification. Lyso was purum grade. Minor amounts of lyso products in the starting DLL material may exist, but they could only contribute to some initial pre-stabilization of the foam films, according to the results reported below. However, to avoid the accumulation of lyso products in the time course of any actual experiment the lipid dispersions were kept under nitrogen at all times after preparation. In this way we could be sure that the measured life-time increase was due to the initially added Lyso and not to some lyso products of DLL oxidative degradation arising during the experiment. Phospholipid dispersions were prepared in 0.15 M NaCl (Merck) at pH 5.2 and the dispersions were shaken mechanically on a vortex mixer for 10 min. In the studies with mixtures the DLL and Lyso dispersions were prepared separately and then mixed. The experiments with mixed PLL/Lyso films were carried out at a constant total concentration of phospholipids (44 $\mu g/cm^3$) by decreasing the DLL and increasing the Lyso concentration in the water dispersions. All studies were performed at 37 °C.

Microscopic foam films of pure and mixed DLL were studied by the method of Scheludko and Exerowa (see Exerowa et al. 1983, 1984; Exerowa and Kashchiev 1986). Experimental studies on the dependence of the formation probability $W(c)$ and lifetime $\tau(c)$ of BFF as a function of phospholipid concentration, c, were performed with an apparatus previously described (e.g. Lalchev 1984; Naydenova et al. 1985, 1987). Basically it includes a capillary with an attached system for BFF formation placed in a thermostrated box and an inverted microscope for reflected light observation. The formation probability at a given concentration, $W(c)$, was obtained as the percentage of foam films showing at least one black spot before rupture in a series of, for example, 100 attempts. The film lifetime, $\tau(c)$, between the appearance of the first black spot and the film rupture was measured with a chronometer.

Results and discussion

The dependence of the formation probability of BFF on the concentration of pure DLL at 37 °C is shown in

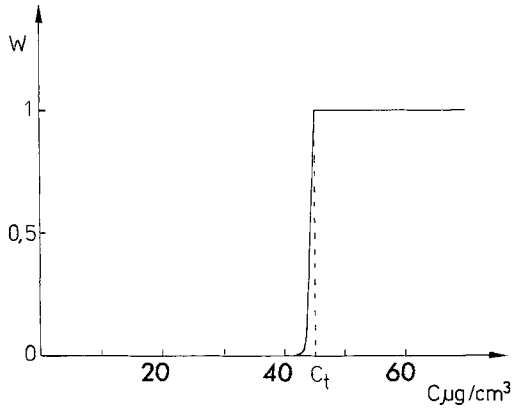

Fig. 2. Formation probability, W as a function of the concentration, c, of dilauroyl lecithin in salt solution (0.15 M NaCl, pH 5.2) at 37 °C. $C_t = 45$ μg/cm³. The curve is based on two experimental points showing the onset and the saturation of $W(c)$ only; the rest of the curve is drawn by eye

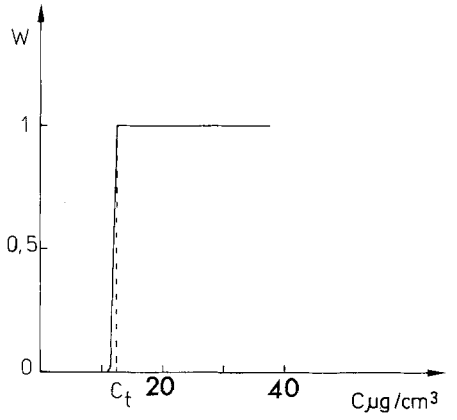

Fig. 3. Formation probability, W as a function of the concentration, c, of palmitoyl lysolecithin in salt solution (0.15 M NaCl, pH 5.3) at 37 °C. $C_t = 12$ μg/cm³. The curve is based on two experimental points showing the onset and the saturation of $W(c)$ only; the rest of the curve is drawn by eye

Fig. 2. It turned out that this dependence featured a pronounced step-like character. W was equal to zero up to 43 μg/cm³ where a single black spot appeared in a 100 attempt series and at the slightly higher concentration of 45 μg/cm³ it became 100%, i.e. all films displayed black spots before rupture. No intermediate points in this very narrow concentration range could accurately be measured, unlike some surfactants (not lipids) (Exerowa et al. 1983) where the concentration range for $W(c)$ increase was much broader. Therefore, Fig. 2 (and Fig. 3) were based on just two experimental points, showing only the onset and the saturation of $W(c)$. In the case of such a step-wise $W(c)$ dependence it is possible to define a threshold concentration, C_t, with respect to BFF formation as the minimum concentration for 100% black spot appearance. In the case of DLL, $C_t = 45$ μg/cm³.

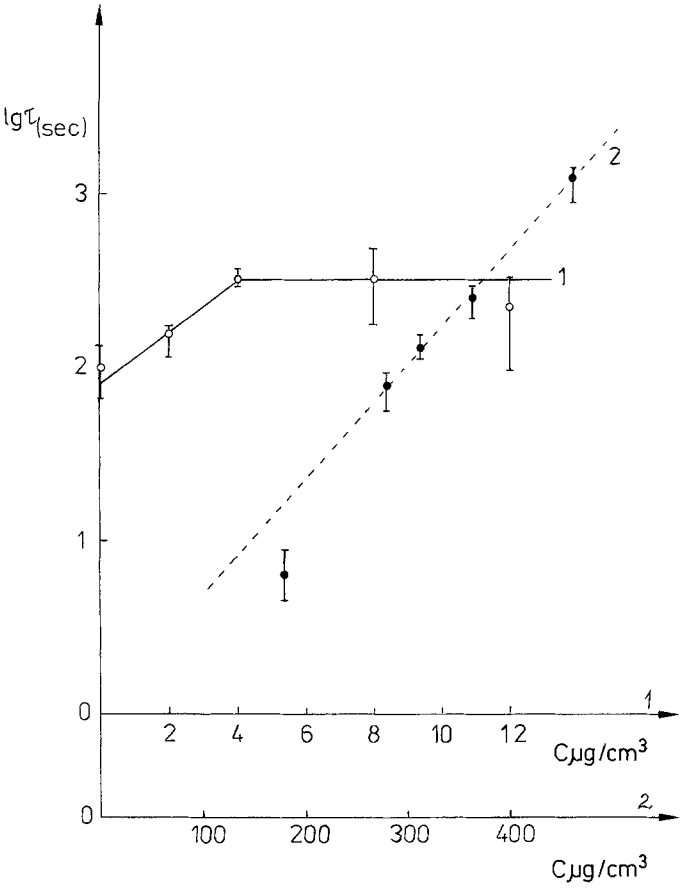

Fig. 4. Comparison of the stabilizing action of lysolecithin on the log(lifetime) of black foam films at constant total concentration of lipids: PLL + Lyso = 44 μg/cm³ in 0.15 M salt solution, pH 5.2, 37 °C (curve 1, abscissa 1, present work) and on the log (waiting time) for membrane fusion (curve 2, abscissa 2, Chernomordik et al. 1985)

The $W(c)$ dependence for the pure Lyso is shown in Fig. 3 and this is also very steep. The Lyso threshold concentration under the same experimental conditions was found to be $C_t = 12$ μg/cm³. The difference in C_t for these two lipids probably reflects, among other factors (e.g. different CMCs, different volume/surface redistribution ratios), the difference in lipid shape asymmetry.

The experiments with mixed DLL/Lyso black foam films were performed at a constant total phospholipid concentration of 44 μg/cm³, just below the C_t of DLL. The log(lifetimes) of the mixed BFFs as a function of the concentration of added Lyso are shown in Fig. 4, curve 1. The initial linear part of this curve means that there is an exponential increase of the lifetime with concentration in the low Lyso concentration range. This exponential increase persists up to about 4 μg/cm³ Lyso where it eventually saturates. Most probably this is due to the fact that volume monomer concentration of Lyso does not increase any more because of reaching the CMC, so that the surface Lyso

concentration is also saturated. Lysolecithin CMCs in the range of 7 to 10.10^{-6} M (i.e. 3.5 to 5 μg/cm^3 with Lyso mol wt of 495.64) have been reported (Haberland and Reynolds 1975; Nakagaki et al. 1986).

This stabilization effect of Lyso is in contrast to the destabilization of a single BLM by Lyso, as found previously (Kaltcheva 1984; Mitov and Kaltcheva 1984). This is the first time that opposite effects of Lyso molecules with respect to the stability of BFF and BLM systems have been observed. This could be an experimental proof that in going from a BLM system to a BFF system the steric asymmetry effect changes its sign.

On the other hand the initial part of curve 1 in Fig. 4 corresponds well to curve 2 in the same figure, confirming the experimental findings about the fusion of two contacting BLMs of phosphatidyl ethanolamine in the presence of increasing lysolecithin concentrations in the bathing solution (Chernomordik et al. 1985). The concentration scale of fusion experiments (scale 2 in Fig. 4) involves much higher than CMC lysolecithin concentrations, presumably because in that case Lyso has been added to the preformed lipid bilayers and quite a different surface/volume redistribution mechanism and redistribution ratio may not be surprising. The exponential increase of the lifetime with the concentration found in both cases means that the energy of the critical defect for both film rupture and monolayer fusion increases linearly with the lysolecithin concentration, as in fact the liquid crystal approach (Petrov et al. 1980) would predict for a common defect structure of the neck type in the limit of low Lyso concentration. On the basis of these results we can conclude that the processes involved in the formation and rupture of black foam films of lipids adequately reflect the onset of close contact and the fusion of two model or two biological membranes during the so-called monolayer membrane fusion.

Similar ideas about foam film stability and monolayer fusion could emerge from the framework of the inverted micellar intermediate concept (Siegel 1984a, b). We believe that it is important to study the relation of that concept to the liquid crystal approach.

Conclusions

From the results presented here we can draw the following conclusions:

1. Owing to the nucleation mechanism of rupture of BFFs, the $W(c)$ curves of DLL and Lyso are step-wise.

2. The lifetime of mixed DLL/Lyso bilayer foam films increases exponentially with Lyso concentration up to the CMC of Lyso, this means that the energy of the critical defect for rupture increases linearly with Lyso concentration in accordance with the nucleation theory of black foam film rupture and to the lipid shape asymmetry concept of the liquid crystal approach.

3. Stabilization of mixed BFFs by Lyso corresponds well to the increased fusion waiting time induced by Lyso in BLM experiments.

4. This finding illustrates that BFFs may be used as an adequate but simple model of membrane fusion. It also provides a means for quick screening of the fusion capacity of different biphilic and hydrophilic admixtures.

Acknowledgements. This work was supported in part by the Bulgarian Ministry of Culture, Science and Education. Thanks are due to Dr. M. S. P. Sansom for reading and improving the manuscript.

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