Eur Biophys J (1995) 23: 433–438

European Biophysics Journal

Lateral mobility of phospholipid molecules in thin liquid films

Z. Lalchev[1], **R. Todorov**[1], **H. Ishida**[2], **H. Nakazawa**[2]

[1] Department of Biochemistry, Faculty of Biology, Sofia University, 8 Dr. Tzankov str., BG-1421 Sofia, Bulgaria
[2] Department of Physiology 2, School of Medicine, Tokai University, Isehara, Kanagawa 259-11, Japan

Received: 5 January 1994 / Accepted in revised form: 27 October 1994

Abstract. The Fluorescence Recovery After Photobleaching (FRAP) method was applied to measure the lateral mobility of the fluorescent lipid analog, dioctadecylindocarbocyanine perchlorate (DiI-C18), in microscopic thin liquid films (Foam Films (FFs)). The foam film structures were comprised of two phosphatidylcholine monolayers adsorbed at air/water interfaces which sandwiched a thin liquid core. Lateral diffusion of the DiI molecules in the plane of the monolayers was determined as a function of the thickness of the thin liquid core of the film between the FF monolayers. The results obtained indicated that the diffusion coefficient was strongly dependent both on the distance between the FF monolayers in the range 4 nm to 85 nm (corresponding to the FF thickness) and on the film type. The applicability of the FRAP method for studying the molecular mobility in phospholipid FFs was demonstrated. Considerable differences in the surface diffusion coefficient of DiI were observed, ranging between 2×10^{-8} cm^2/s and 22×10^{-8} cm^2/s in so called yellow, gray, common black and Newton black FFs. The effect of the presence of polyethylene glycol (PEG-400) in the liquid core of lecithin FFs on surface diffusion was also studied. The surface diffusion results from the FF studies were compared with data from black lipid membranes (BLMs). These structures are related in thickness terms but the molecular orientation in FFs is the reverse of that in BLMs.

Key words: Lecithin – Foam films – Lateral diffusion – FRAP – PEG

Introduction

It is known that the lateral mobility of cell surface constituents plays an important role in controlling the response of the cell to its external environment. Several membrane systems have been investigated experimentally to determine molecular mobility using the Fluorescence Recovery After Photobleaching (FRAP) method (Axelrod et al. 1976, 1983; Angelides et al. 1988). The FRAP method has been used to measure surface diffision in membranes of living cells (e.g. Schlessinger et al. 1976, 1977; Ishida et al. 1993; Greenberg and Axelrod 1993; Eldridge et al. 1980) and in model membrane systems, for instance in phospholipid monolayers (e.g. Beck and Peters 1985; Tombs and Thompson 1990), bilayer lipid membranes (BLMs) (Fahey et al. 1977).

Recently, the papers of several authors have extended for the first time the application of the FRAP method to bilayer thin liquid films, such as bilayer foam films (Clark et al. 1990a; Lalchev et al. 1991a). Clark and coworkers were the first to report FRAP measurements of surface diffusion of the fluorescent probe, 5-N-(octadecanoyl) aminofluorescein incorporated in foam films (FFs) stabilized with SDS (Clark et al. 1990a). This was followed by measurements in protein stabilized FFs (Clark et al. 1990b; Coke et al. 1990) and in phospholipid FFs (Lalchev et al. 1991a). These reports confirmed the applicability of the FRAP method in the study of FF systems.

Foam films are thin liquid films of varying thickness (Fig. 1) consisting of two adsorbed monolayers of surface active molecules at the upper and lower air/water interfaces, separated by a liquid core. Generally, they are self-associated, ordered systems, existing in unstable, metastable and stable states. Metastable and stable FFs have lifetimes ranging from several hours to days and can be formed in vitro and in vivo in many systems in nature (e.g. in foams, emulsions, at contacts between cell membranes etc.) They have been investigated intensively over several decades (e.g. Mysels et al. 1959; Scheludko 1967; Ivanov 1988; Exerowa et al. 1992). Recently, black foam films (BFFs, Fig. 1, schematic 3 and 4) stabilized by phospholipids have acquired special interest. These structures exhibit some properties similar to BLMs including thickness, refractive index, stability etc., but the molecular orientation in the phospholipid BFFs (Fig. 1, schematic 4) is the reverse of that observed in BLMs. Previous studies of pure phospholipid BFFs (Lalchev 1984) and of mixed lipid-pro-

---

Correspondence to: Z. Lalchev

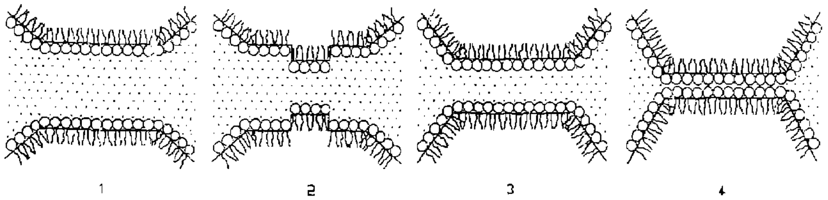

Fig. 1. A schematic diagram of lecithin foam films (FFs) of different types: 1 – Equilibrium thick foam films which appear yellow or gray in colour when viewed in reflected light under a microscope, the thickness of the liquid core (in the range 30–90 nm in this study) between the monolayers is regulated by the electrolyte concentration; 2 – FF with black spot signifying local thinning within the film; 3 – Common black foam film (film thickness (h) circa 13 nm); 4 – Newton black foam film (h=8 nm)

tein BFFs obtained from human or animal lung surfactants and human or animal amniotic fluids (Lalchev 1984; Exerowa et al. 1981; Exerowa et al. 1984) have shown that they can be used as model systems for the investigation of inter membrane interactions (Exerowa and Lalchev 1986), cell fusion (Naydenova et al. 1990), lung physiology (Exerowa et al. 1986; Lalchev et al. 1992; Scarpelli et al. 1994) and coalescence phenomena in food emulsions and foams (Clark et al. 1989; Clark and Wilde 1992) These reports have also shown that spontaneously formed lipid and protein BFFs are very stable (usually more so than BLMs) and convenient for systematic investigations by FRAP (Lalchev et al. 1991 a, 1994).

In contrast to SDS and protein-stabilized FFs, no systematic data have been published reporting lateral diffusion in phospholipid FFs. Determination of the lateral diffusion properties of the interfacially adsorbed lipid analog DiI-C18 (1, 1′-dioctadecyl-3, 3, 3′, 3′-tetramethylindocarbo-cyanine perchlorate) in FFs composed of phosphatidylcholine (lecithin) molecules is the basis of this study. The FRAP method was applied to lecithin FFs of different thicknesses in order to investigate the dependence of the lateral diffusion on the distance separating the film monolayers in the range 4 nm–85 nm. Lateral diffusion in two types of black films, referred to as common and Newton black foam films (Fig. 1, schematic 3 and 4 respectively) and the effect of PEG-400 in the interlamellar liquid were studied. The film thickness and type investigated in this study were controlled by the level of electrolyte (NaCl) added to the lipid dispersions and the resultant change in the film thickness was measured by an interferometric technique. Use of electrolyte concentration as a means to control film thickness is well established and the mechanism of this effect is explained by the DLVO theory (Deryagin and Landau 1941; Verwey and Overbeek 1948).

Materials and methods

L-à-Phosphatidylcholine type II-S (P 5638) was obtained from Sigma Chemical Co. and further purified according to the method of Kagawa and Racker (1971). DiI-C18 (1,1′-dioctadecyl-3, 3, 3′, 3′-tetramethylindocarbocyanine perchlorate) obtained from Molecular Probe Inc. was dissolved in dimethyl sulfoxide (DMSO) to form a stock solution of concentration 3 mg/ml. An aliquot (10 μl) of this solution was added to 1 ml of a 3.3 mg/ml lecithin dispersion. Under these conditions the lecithin:fluorophore molar ratio was approximately 150:1.

Polyethylene glycol of average molecular weight 400 (PEG-400) was obtained from Sigma Chemical Co. and was added to the lecithin dispersion to give a final concentration of up to 30% (v/v). An appropriate amount of NaCl (Merck) was added to the lipid dispersions to obtain the desired FF type. Gray FFs, characterized by thicknesses between 90 nm and 30 nm were obtained from dispersions containing <9 mM NaCl. The dependence of the film thickness on the electrolyte concentration in the dispersions was shown to agree with the DLVO-theory (Deryaguin and Landau 1941; Verwey and Overbeek 1948). Common black and Newton black FFs were obtained from dispersions containing 136 mM and 940 mM NaCl respectively.

Foam films for study by FRAP were formed in a specially constructed chamber of dimensions 10×10×1.5 cm as described previously (Lalchev et al. 1991 a, Lalchev et al. 1991 b). The thin liquid film was formed and supported in a glass annulus, (diameter 0.4 cm) contained within a quartz cuvette (dimensions 1×1×2 cm). The latter is housed in the thermostatted body of the chamber. The film was formed by reduction of the liquid volume of the droplet contained within the annulus by suction using a sensitive pump until a planar film was formed in the center of the annulus (Fig. 1, schematic 1). Typically, the diameter of the FF ranged between 100 and 500 μm and its dimensions were regulated by the pump.

Lateral diffusion measurements were performed on FF contained in the film chamber by FRAP using an argon ion laser (488 nm), as described previously (Ishida et al. 1993). A low intensity laser beam was focused on the FF using a long working distance objective. The fluorescence signal from the spot was monitored both before and after a brief, intense bleaching laser pulse. During the monitoring stage the laser beam (uniform intensity profile) was attenuated using a neutral density filter. Recovery curves (number of data curves, n=10–40) for each FF type were averaged and analyzed by nonlinear least-squares fitting to an expression given by Axelrod et al. (1976) using a microcomputer. The diffusion coefficient (D) was calculated from $D\ (\mathrm{cm}^2/\mathrm{s}) = \omega^2\ (\mathrm{cm})/4t_{1/2}\ (\mathrm{s})$, where ω is the radius of the laser spot (4.5 μm) and $t_{1/2}$ is the half-life of the fluorescence recovery. The D values of four different FF types (shown in Fig. 1) were determined in this study. The film type was estimated by its thickness (h, (nm)) and colour using an inverted microscope (Epityp 2, Carl-Zeiss-Jena) in epi-illumination mode typically equipped with an objective lens of magnification 50×. The FF thicknesses was calculated from the intensity of reflected light from both film surfaces using an interferometric apparatus described in several papers (e.g. Scheludko 1976; Exerowa and Kashchiev 1986). We used the "equivalent water" model where the film is considered homogenous with a refractive index equal to that of the aqueous dispersions from which the film is obtained.

Surface tension γ (mN/m)-time isotherms at the air/water interface for the lipid dispersions were measured to determine the rate of adsorption of lecithin molecules at the interfaces of the FFs. The surface tension was measured with an accuracy of 0.1 mN/m by an automatic balance (Wilhelmy Tensiometer, Biegler Electronic, Austria) according to the method of Wilhelmy. The adsorbed monolayers were formed by introducing the lecithin dispersions into a Teflon trough (diameter 3 cm and depth 1.7 cm) and the γ-t curves were monitored using a Pt plate.

Results and discussion

A number of control experiments were performed and experimental parameters defined prior to initiation of the FRAP experiments to establish optimal conditions for this investigation.

Firstly, the probability (W) of formation of stable black FFs as a function of lecithin concentration (C) was determined from the ratio $\Delta N/N$ (Exerowa and Kahchiev (1986)), where ΔN is the number of cases in which stable black FFs were formed and N is the number of all trials. The results (not shown here) revealed that stable FFs were always formed at lecithin concentrations above 80 µg/ml (W=1). Since the stability of the lecithin FF for the duration of the FRAP measurements was critical, lecithin concentrations above the 80 µg/ml threshold concentration, specifically 0.33 and 3.3 mg/ml, were selected.

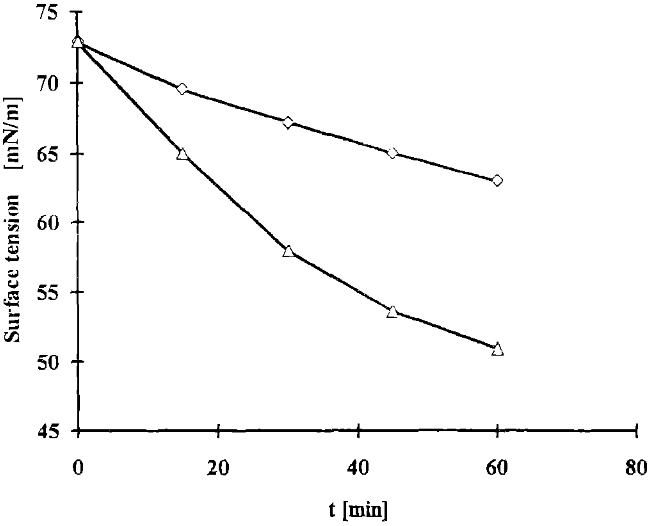

Fig. 2. Dependence of the surface tension γ (mN/m) of lecithin dispersions at the air/water interface on the adsorption time t (min) at different concentrations. T=24 °C, C_{el}=136 mM NaCl. ◇, 0.3 mg/ml; △, 3.3 mg/ml

Secondly, the surface properties of lecithin solutions in this concentration range were partially characterized. Figure 2 shows the dependence of the surface tension γ (mN/m) on the adsorption time of lecithin molecules at the air/water interface in the presence of DiI. The first photobleaching measurement on a fresh film in this study was always performed 30 min after introduction of the droplet of lecithin solution into the annulus in the film chamber. This equilibration time was necessary for several reasons; to ensure vapour saturation in the film chamber to minimize evaporation from the film, to ensure adequate adsorption of lecithin molecules at the air-water interface and to ensure good thermal equilibration. The γ-t curves in Fig. 2 were obtained for two lecithin concentrations which had the capability of supporting the formation of stable FFs after 30 min equilibration. It is evident that the surface tension decrease achieved after 30 min equilibration of the sample containing a lecithin concentration of 0.3 mg/ml is much smaller than that for the sample containing 3.3 mg/ml. This indicates some differences in the surface packing of adsorbed lipid. It is notable that the γ-t curves were not affected by the inclusion of the fluorophore DiI at concentrations equivalent to those required for the FRAP experiment. Further evidence in support of this finding comes from the observation that incorporation of DiI molecules into the lecithin monolayers of the foam film did not alter the features of the W/C curve or the film stability measured by its lifetime. The increase in the density of molecular packing at the interfaces of FFs is likely to be small with increasing concentration above the threshold concentration for stable FF formation (i.e. 80 µg/ml), since the latter requires an almost packed monolayer. Thus, FRAP measurements were performed on FFs formed from 0.33 and 3.3 mg/ml lecithin solutions.

Table 1 summarizes the results obtained by the FRAP method for the recovery half times ($t_{1/2}$) and the lateral diffusion coefficients (D) of DiI molecules in FFs of different types. The film type was distinguishable both by the film colour and thickness. The film colour depends on the film thickness since a typical interference picture was observed under the microscope of the light reflected from both film surfaces. Significant differences between the values of D were obtained for very thick FFs (yellow in colour,

Table 1. Lateral diffusion coefficients (D) of DiI molecules in lecithin foam films of different types

Lecithin [mg/ml]	NaCl [mM]	Film color and thickness [nm]	n[a]	$t_{1/2}$±(SD) [s]	D×10^8 [cm^2/s]
3.30	–	Yellow; h>80	22	0.23 (0.04)	22.3 (52.8)[b]
0.33	3.3	Gray; h=45–55	38	0.52 (0.04)	9.7 (44.0)
0.33	9.5	Dark gray; h=25–35	11	0.63 (0.10)	8.0 (23.0)
0.33	136	Common black; h=12–14	34	1.00 (0.05)	5.0 (8.6)
0.33	940	Newton black; h~8	18	2.25 (0.16)	2.2 (18.0)

[a] n is the number of FRAP experiments with the same FF, relative uncertainty in D in this case is less than ± 17.3%

[b] the numbers in the brackets of this column show the uncertainty (%) of the mean value of D obtained from 3 different film preparations of equivalent thickness

h>80 nm) and for dark gray FFs (h circa 30 nm). A further decrease in D was observed in common black films ($D=5\times10^{-8}$ cm^2/s). The latter films, have an equivalent water thickness of approximately 13 nm and consist of a free water layer between two adsorbed layers. The thickness of the free water layer can be calculated by "the three layer" model commonly applied to these systems. The value of the lateral diffusion coefficient in the Newton black films, which are characterized by a thickness of approximately 8 nm (the thinnest foam bilayers) and which contain no free water layer between the monolayers, was a factor of two lower than that in the common black films. Since the decrease of the film thickness reflects the decrease of the free water layer thickness (at constant temperature), the results reported above support the conclusion that reduction in the liquid core thickness of the FFs leads to slower lateral molecular diffusion. In other words, the attractive and repulsive molecular interactions present in the film have an influence on the lateral mobility of molecules in the adsorbed monolayer of the FF as detected by changes in D.

The above result may be of profound significance in relation to membranes. The orientation of the adsorbed lipids at the interfaces of the FF allows this structure to be considered as a model for interactions that may occur between the outer leaflets of two close approaching cell membranes. Our observation establishes a link between intermembrane interactions (which are dependent on the distance separating the outer surfaces of the two membranes) and intramembrane interactions (i.e. the lateral movement experienced by the phospholipid molecules that constitute the membrane) and their potential interdependence. One can only speculate about the importance of such phenomena in a wide range of biological processes including cell-cell interactions, cell fusion and fission phenomena, etc.

The dependence of the lateral diffusion coefficient (D) on film thickness (h) in the range 30–100 nm measured to an accuracy of 0.5 nm is shown in Fig. 3. The values of D which correspond to the Newton and common black films respectively are given for comparison (2.2×10^{-8} cm^2/s and 5.0×10^{-8} cm^2/s, see Table 1). There is a statistically significant difference ($P<0.001$) between these values which could be used to distinguish between the two types of the black films. In the range of FF thicknesses between 30 and 100 nm, the values of D varied from approximately 8 to 20×10^{-8} cm^2/s. It should be noted that the variability in D in a given film and in different FFs formed from solutions of the same composition increased with film thickness (see Table 1). This effect is greatest in yellow films with h>80 nm and may be connected with the homogeneity and mobility of the adsorbed layers of the FF (i.e. the thicker film surfaces are more mobile and generate more heterogeneity in the laser spot area during the time course of the FRAP measurements). The clear trend observed in Fig. 3 is that as the FFs increase in thickness, there is a corresponding increase in the lateral diffusion coefficient of DiI in the adsorbed layer. Ultimately, the latter will approach the characteristic value for D of DiI in lecithin monolayers at a macroscopic air/water interface above an infinitely thick subphase. Unfortunately, it was impossible to perform FRAP measurements on FFs with thicknesses > 100 nm, due to the intrinsic ionic strength (although small) of the lecithin dispersions when prepared in double distilled water without NaCl addition.

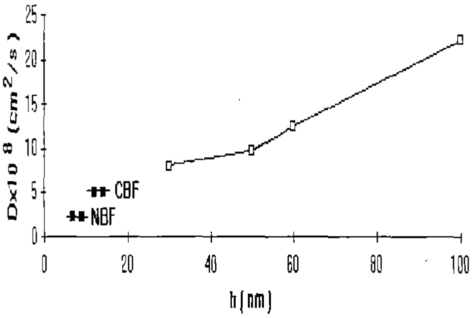

Fig. 3. Dependence of the lateral diffusion coefficient (D, cm^2/s) of surface adsorbed DiI molecules on foam film thickness (h, nm). Lecithin concentration = 3.3 mg/ml. Electrolyte concentrations (NaCl) were varied as described in Material and methods. T=24 °C

The availability of data for the lateral diffusion of DiI in phospholipid FFs is currently limited to this report so it is interesting to compare the D value observed with Newton black foam film (considered as a water-free lipid bilayer structure) with those reported for BLM structures in the literature (e.g. Fahey et al. 1977). Under similar conditions of phospholipid (egg lecithin), fluorescent probe (DiI) and temperature (24 °C), the value of D in Newton black films was one order of magnitude lower ($D=2.2\times10^{-8}$ cm^2/s, Fig. 3) than that characteristic for BLMs. Using the fluorescence correlation spectroscopy method, Fahey et al. (1977) reported D values between 1.7 and 4.4×10^{-7} cm^2/s depending on the BLM preparation technique. Direct comparison of the data are difficult as different measurement methods were used and the NaCl concentration was not stated for the BLM preparations. Nevertheless, assuming that the real thicknesses of both structures are essentially equivalent, the difference in the D values could relate to the different orientation of the monolayers with respect to each other in these two bilayer structures (see Fig. 1, schematic 4). More detailed comparison must await a systematic study of both membrane structures by the same method and under the same conditions.

The FRAP data curves for the different types of a lecithin foam film are shown in Fig. 4. The difference between the shape of the curves of common black and Newton black films (curve 3 and 4 in the insert, see also Fig. 1, schematic 3 and 4 respectively) is clearly seen and was obviously caused by the change in the film type. On the other hand, due to the much bigger thicknesses of the yellow FFs (h>80 nm, see Fig. 1, schematic 1), their averaged recovery curve (Fig. 4, curve 1 in the insert) differ strongly from that of the black films and dark gray ones (Fig. 4, curve 2). In addition, it should be noted that complete fluorescence recovery was observed in all the film types studied, indicating the absence of an immobile fraction in the laser spot area.

It was of interest to investigate whether the value of D varied with the positioning of the laser spot within the phospholipid FF to ascertain whether the phospholipid

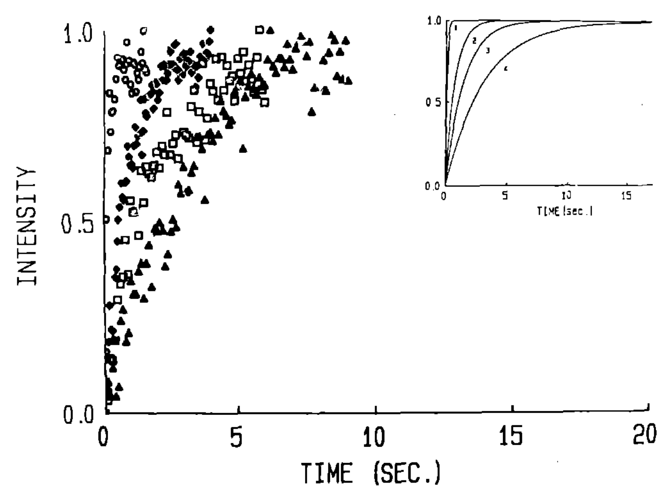

Fig. 4. Experimental FRAP data curves obtained for lecithin foam film (FF) of different types. ○, yellow FF; ◆, dark gray FF; □ common black FF; ▲ Newton black FF. The fluorescence intensities of the different film types are normalized. The intensity was detected every 0.1 seconds. Insert: A drawing of the FRAP curves for the four film types. The curve for each type is obtained from the averaged FRAP data of a number of experiments (n) with the same FF and with 3 different film preparations of equivalent type (n is indicated in Table 1). 1 – yellow FF, 2 – dark grey FF, 3 – common black FF and 4 – Newton black FF. T=24 °C

monolayers in the FF were homogenous. FRAP measurements were made as a function of laser spot position and FF radius (Table 2). The values of D measured within a growing Newton spot in the FF (see Fig. 1, schematic 2) and these obtained immediately after the complete Newton film formation (no waiting for equilibration) did not exhibit significant differences (4.2 to 4.0×10^{-8} cm^2/s). Further studies did not detect a position dependence of D in the FF except in cases where film drainage was evident whereupon a slight change in D was observed. Decreasing the film radius without changing the laser spot position led to a slight increase in D (Table 2, lines 2 and 3). The latter effect was probably due to the influence of effects of the film periphery on D. The above results showed that the sensitivity of the FRAP method allows study of heterogeneity and dynamics in Newton FFs of phospholipids event though these structures appear homogeneous in terms of thickness and film colour.

The effect of adding polyethylene glycol (PEG-400, 30% v/v) to common black films was studied (Table 2). A slight dependence of the D of DiI in common black FFs was observed in relation to the position of the laser spot in the film. The addition of PEG resulted in a decrease in D from about 5 to 3×10^{-8} cm^2/s (Table 2). We suggested that the decrease in D could be for several reasons. Firstly, PEG increased the bulk viscosity of the interlamellar solution (see Fig. 1, schematic 3). A second possibility is that the PEG molecules penetrate into the adsorbed lipid monolayers of the FF resulting in a change in the molecular interactions and a consequent change in the film thickness. We found in a separate investigation that both the film thickness decreased and sharp change occurred in the FF properties when PEG was present between the film monolayers (data not shown). More detailed investigations into the dependency of phospholipid lateral diffusion on the molecular weight and concentration of PEG (known as a promoter of cell fusion), is required to clarify the nature of these effects.

Conclusions

The following conclusions may be drawn from the results: (i) The FRAP method can be applied to study the dynamics of lipid molecules at the interfaces of thick (yellow and gray) and thin (black) foam films. (ii) Lateral diffusion in lipid foam films depends strongly both on the distance between the film monolayers in the case of thick films and on the type of black foam films. (iii) Phospholipid FFs coupled with the FRAP method can be used as a model system for the study of the lateral lipid mobility in the plane of the thin liquid films formed at the point of contact between two cell membranes (i.e. a model for cell fusion, membrane-membrane interactions etc.). This approach benefits from the ease of formation of different types of the film and regulation of the distance between the film monolayers simply by altering the electrolyte concentration according to the DLVO-theory (Deryaguin and Landau 1941; Verwey and Overbeek 1948).

Acknowledgement. Z. Lalchev thanks Dr. M. Tsuda, M. Yamamura, Chairman, Dept. of Biochemistry, Dr. T. Nakae, Chairman, Dept. of Cellular Inf. Sci., in Tokai University, School of Medicine for their

Table 2. Lateral diffusion coefficients (D) of DiI molecules in lecithin common and Newton black foam films with radius of 3×10^{-2} cm

Sample [mg/ml]	NaCl [mM]	Film type	Laser spot position in the film	$t_{1/2} \pm SD$ [s]	$D \times 10^8$ [cm^2/s]
Lecithin	940	Newton	in growing black spot[a]	1.19 ± 0.16	4.2
(c=0.33)	940	black	averaged from different regions	1.26 ± 0.13	4.0
	940	films	averaged from different regions	1.07 ± 0.11	4.7[b]
	300	Common	averaged from the center	0.97 ± 0.07	5.2
	300	black	averaged from thge periphery	1.02 ± 0.07	5.0
+ PEG-400 (30 vol.%)	300	films	averaged from different regions	1.70 ± 0.17	3.0

[a] See Fig. 1, scheme 2

[b] This value was obtained at two-fold decrease of the film radius

support and assistance throughout the course of this work. Thanks also to Dr. D. Clark, Institute of Food Research for discussions and assistance in preparing the manuscript.

References

Angelides KJ, Elmer LW, Loftus D, Elson E (1988) Distribution and lateral mobility of voltage-dependent sodium channels in neurons. J Cell Biol 106:1911–1925

Axelrod D (1983) Lateral motion of membrane proteins and biological function. J Membr Biol 75:1–10

Axelrod D, Koppel D, Schlessinger J, Elson E, Webb W (1976) Mobility measurement of analysis of fluorescence photobleaching recovery kinetics. Biophys J 16:1055–1069

Beck K, Peters R (1985) In: Bayley P, Dale R (eds) Spectroscopy and the dynamics of molecular biological systems. Academic Press, New York London, p 177

Clark D, Wilde P (1992) In: Phillips G, Wedlock D, Williams P (eds) Gums and stabilisers for the food industry. Oxford Press, Oxford, p 343

Clark D, Mackie A, Smith L, Wilson D (1989) In: Bee R, Richmond P, Mingins J (eds) Food Colloids. R Soc Chem Special Publ No 75:97

Clark D, Dann R, Macki A, Minings J, Pinder A, Purdy P, Russell E, Smith L, Wilson D (1990a) Surface diffusion in sodium dodecyl sulfate-stabilized thin liguid films. J Colloid Interface Sci 138:195–206

Clark D, Coke M, Mackie A, Pinder A, Wilson D (1990b) Molecular diffusion and thickness measurements of protein-stabilized thin liguid films. J Colloid Interface Si 138:207–219

Coke M, Wilde P, Russell E, Clark D (1990) The influence of surface composition and molecular diffusion on the stability of foams formed from protein/surfactant mixtures. J Colloid Interface Sci 138:489–497

Deryaguin B, Landau L (1941) Theory of the stability of strongly charged lyophobic sols. Acta Physiocochim URSS 14:633–662

Elridge C, Elson E, Webb W (1980) Fluorescence photobleaching recovery measurements of surface lateral mobilities on normal and SV40-transformed mouse fibroblasts. Biochemistry 19:2075–2079

Exerowa D, Kashchiev D (1986) Hole-mediated stability and permeability of bilayers. Contemp Phys 27:429–461

Exerowa D, Lalchev Z (1986) Bilayer and multilayer foam films: model for study of the alveolar surface and stability. Langmuir 2:668–671

Exerowa D, Lalchev U, Marinov B, Ognianov K (1981) Method for assessment of fetal lung maturity. Bulgarian Institute for Inventions, Authorship Cert No 50291

Exerowa D, Lalchev Z, Kashchiev D (1984) Stability of foam lipid bilayers of amniotic fluid. Colloid Surf 10:113–121

Exerowa D, Lalchev Z, Marinov B, Ognyanov K (1986) Method for assessment of fetal lung maturity. Langmuir 2:664–668

Exerowa D, Kashchiev D, Platikanov D (1992) Stability and permeability of amphiphile bilayers. Adv Colloid Interface Sci 40:201–256

Fahey P, Koppler D, Barak L, Wolf D, Elson E, Webb W (1977) Lateral diffusion in planar lipid bilayers. Science 195:305–306

Greenberg M, Axelrod D (1993) Anomaously slow mobility of fluorescent lipid probes in the plasma membrane of the yeast Saccharomyces cerevisiae. J Membr Biol 131:115–127

Ishida H, Hirota Y, Nakazawa H (1993) Effect of sub-skinning concentrations of saponin on intracellular Ca2+ and plasma membrane fluidity in cultured cardiac cells. Biochim Biophys Acta 1145:58–62

Ivanov I (1988) Thin liquid films. Marcel Dekker, New York

Kagawa H, Racker M (1971) Partial resolution of the enzymes catalyzing oxidative phosphorylation. J Biol Chem 246:5477–5487

Lalchev Z (1984) Free liquid films of lipid-proteins mixtures. PhD Thesis. Bulgarian Academy of Sciences, Sofia

Lalchev Z, Ishida H, Nakazawa H (1991a) Fluorescence photobleaching recovery measurements on lecithin foam films. In: Jennings B, Stoylov S (eds) Colloid and Molecular Electro-Optics 1991. Institute of Physics Publishing, Bristol and Philadelphia, pp 239–244

Lalchev Z, Alexsandrov N, Todorov R, Exerowa D (1991b) Chamber for formation and investigation of thin liquid films. Bulgarian Institute for Inventions, Patent No 92610

Lalchev Z, Christova Y, Todorov R, Alexandrov V, Stoichev P, Petkov R (1992) Alterations of biochemical and physicochemical quantities of pulmonary lavages attending halothane and penthrane treatment in rats. Appl Cardiopulmon Pathophys 4:315–322

Lalchev Z, Wilde P, Clark D (1994) Surface diffusion in phospholipid foam films. J Colloid Interface Sci 167:80–86

Mysels K, Shinoda K, Frankel S (1959) Soap films. Pergamon Press, New York

Naydenova S, Lalchev Z, Petrov A, Exerowa D (1990) Pure and mixed lipid black foam films as models of membrane fusion. Eur Biophys J 17:343–347

Scarpelli E, Lalchev Z, Todorov R, Exerowa D, Mautone A (1994) Black film formation by three surfactant preparations: characteristics and significance. J Appl Physiol (submitted for publication)

Scheludko A (1967) Thin liquid films. Adv Colloid Interface Sci 1:391–464

Schlessinger J, Koppel D, Axelrod D, Jacobson K, Webb W, Elson E (1976) Lateral transport on cell membranes: Mobility of concanavalin A receptors on myoblasts. Proc Natl Acad Sci, USA 73:2409–2413

Schlessinger J, Axelrod D, Koppel D, Webb W, Elson E (1977) Lateral transport of a lipid probe and labeled proteins on a cell membrane. Science 195:307–308

Tombs M, Thompson K (1990) Slow rotational mobilities of antibodies and lipids associated with substrate-supported phospholipid monolayers as measured by polarized fluorescence photobleaching recovery. Biophys J 58:413–428

Verwey E, Overbeek J (1948) Theory of the stability of lyophobic colloids. Amsterdam, Elsevier