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PRESSURE VARIATION OF THE LATERAL DIFFUSION IN LIPID BILAYER MEMBRANES

HANS-JÜRGEN MÜLLER and HANS-JOACHIM GALLA

Institut für Organische Chemie und Biochemie, Technische Hochschule Darmstadt, Petersenstrasse 22, D-6100 Darmstadt (F.R.G.)

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A pressure-induced decrease of the lateral diffusion in pure and cholesterol containing phosphatidylcholine bilayer membranes has been determined by the excimer formation technique using pyrene as probe molecule. The experimental results at pressures up to 150 bars are described satisfactorily by the free volume theory of a molecular transport in liquids. A pressure increase of extrapolated 575 bars decreases the lateral diffusion of lipids by a factor of two in pure dipalmitoylphosphatidylcholine membranes. Higher pressures are necessary to induce the same effect in cholesterol containing membranes. This result is interpreted by the condensing effect of cholesterol in fluid bilayer membranes.

Lateral diffusion of lipid and protein molecules in lipid bilayer membranes has been the subject of extensive investigations [1] employing different experimental techniques. Electron paramagnetic resonance [2] and fluorescence redistribution after photobleaching (FRAP) [3] were used to determine the diffusion over long distances following the influx of probe molecules into volumes predetermined by a macroscopic concentration gradient of the probe. Other, electron paramagnetic resonance [4,5] and fluorescence techniques [6,7] were developed based on the spectroscopic analysis of diffusion controlled collisions of probe molecules that were incorporated into the lipid bilayer membrane. One powerful method of the latter type is the excimer formation technique [8]. From the association rate of lipophilic excimer probes one obtains the coefficients of the lateral self diffusion of lipids in artificial and natural bilayer membranes [9]. Typical values of the diffusion coefficients between 10^{-7} and 10^{-8} cm^2/s can be determined by this method.

Two models exist for the theoretical treatment of the problem of lateral diffusion in quasi-two-dimensional systems like membranes. One is the hydrodynamic model by Saffman and Delbrück [10] which describes the diffusion process by Brownian motion in a sheet of a viscous medium. A second model developed by Galla et al. [11] in connection with the excimer formation technique is based on the application of the free volume theory of a molecular transport in liquids [12] which yields an expression for the coefficient of the lateral diffusion:

$$D \sim \exp\left(-\frac{v^*}{v_f}\right)$$

$$= \exp\left(-\frac{v^*}{\bar{v}_m \cdot \alpha \cdot (T - T_0) - \bar{v}_P \cdot \beta \cdot \Delta P}\right) \quad (1)$$

where v^* is a critical volume large enough to permit a diffusive displacement and v_f is the actual free volume; note that D is zero until v_f exceeds v^*. At constant pressure the free volume is equivalent to the total thermal expansion $v_f = \alpha \cdot \bar{v}_m(T - T_0)$, where α is the thermal expansion coefficient, $\bar{v}_m$ is the mean molecular volume, T is the temperature and T_0 a temperature below which the free volume disappears (e.g. the lipid pre-transition

temperature). Now we consider the pressure variation of v_f leading to Eqn. 1 where $\bar{v}_P$ is the mean molecular volume for the pressure increment ΔP and β is the mean compressibility. For a two-dimensional system like membranes v^*, $\bar{v}_m$ and $\bar{v}_P$ have to be replaced by the corresponding areas a^*, $\bar{a}_m$ and $\bar{a}_P$.

The diffusion coefficients can be determined from the fluorescence intensities of the excimer, I', and the monomer, I, of pyrene molecules incorporated into the membrane. The intensity ratio I'/I is a measure for the diffusion controlled collision process of pyrene molecules incorporated into lipid bilayer membranes. The jump frequency, ν_j, in a lipid matrix is directly related to the intensity ratio by $\nu_j \sim \text{const} \cdot I'/(I \cdot \tau_0')$ where τ_0' is the excimer life time, which has to be measured separately. The coefficient of the lateral diffusion, D, is related to ν_j by $D = \nu_j \cdot \lambda^2/4$, where λ is the length of one diffusional step which is given by the average distance of two neighboured lipid molecules (λ = 0.8 nm). For further details see Refs. 8, 11, 13 and 14.

Fluorescence spectra were taken with a Fluorescence Spectrometer equipped with two sets of monochromator/photomultiplier systems arranged perpendicular to the irradiation light path. A complete description of the experimental layout is given elsewhere [14]. The arrangement allowed the measurements of fluorescence spectra after irradiation of pyrene at λ = 335 nm. We were also able to measure the fluorescence intensity of the excimer emission (I') at 470 nm and of the monomer emission (I) at 394 nm simultaneously. Calculation of the ratio I'/I by a micro-computer permitted the measurement of I'/I concurrently with temperature.

The samples were kept in 1 cm quartz cuvettes in a temperature controlled specially constructed metal block. Temperature was measured by a thermocouple in the sample. The metal block was equipped with pressure resistant excitation and emission quartz windows. The desired pressure was attained from a helium source pressurized to 200 bars. Before each measurement the metal block containing the fluorescence cuvette was pressurized and vented several times to remove residual air from the system.

Dipalmitoylphosphatidylcholine (DPPC), cholesterol and pyrene were obtained from Fluka (Neu-Ulm, F.R.G.) and were used without further purification. Lipids, cholesterol and pyrene were dissolved in chloroform in the desired concentration and dried as a lipid film on the wall of a glass flask in a nitrogen stream at a temperature above the corresponding phase transition temperature. After evaporation of the solvent under vacuum the lipids were dispersed in $2 \cdot 10^{-3}$ M CsCl solution by sonification with a Branson Sonifier for 2 min at 30 W using a microtip. The temperature was kept above the phase transition temperature. The lipid concentration was 1 mg/ml, the pyrene concentration was 20 mmol/mol lipid.

We have investigated the pressure dependent decrease of the lateral diffusion by the excimer formation technique in dipalmitoylphosphatidylcholine bilayer membranes containing different amounts of cholesterol. Typical phase transition curves for membrane preparations containing a mole fraction of ρ = 0.02 or ρ = 0.17 of cholesterol

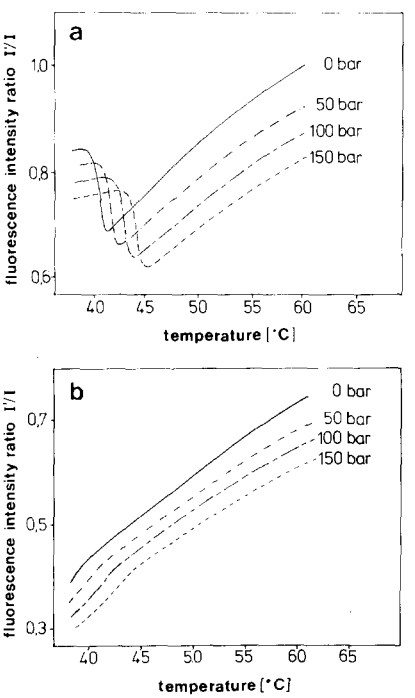

Fig. 1. Phase transition curves at different external pressures obtained from the temperature dependence of the excimer to monomer fluorescence intensity ratio I'/I of pyrene incorporated into dipalmitoylphosphatidylcholine membranes containing cholesterol in a mole fraction of (a) ρ = 0.02, (b) ρ = 0.17.

are shown in Fig. 1a and 1b at various external pressures. The lipid phase transition which is characterized by decrease of the excimer to monomer intensity ratio I'/I, is shifted to higher temperatures by the application of pressure. Above the lipid phase transition temperature one clearly observes a decrease in the excimer to monomer intensity ratio I'/I with increasing pressure. From the intensity ratio I'/I we have calculated the diffusion coefficients at 55°C. The excimer life time τ_0' was measured for each membrane preparation and did not change in the applied pressure range. Typical values for τ_0' were 60–80 ns, typical jump frequencies are in the order of $\nu_j \sim 10^8\ s^{-1}$ which corresponds to diffusion coefficients of $D \sim 2 \cdot 10^{-7}\ cm^2/s$ (see Ref. 11 for further data).

Here we are only dealing with the relative changes. The pressure effect on the diffusion coefficients determined in membrane preparations of different cholesterol contents is shown in Fig. 2. A linear decrease of $\ln(D/D_0)$ with pressure was observed. D_0 is the diffusion coefficient at $P = 0$ bar and D the diffusion coefficient determined at the given pressure. The relative change of the diffusion coefficient for a given pressure increment was found to be smaller for membrane preparations containing cholesterol.

In the pure dipalmitoylphosphatidylcholine membranes the diffusion coefficient is reduced to

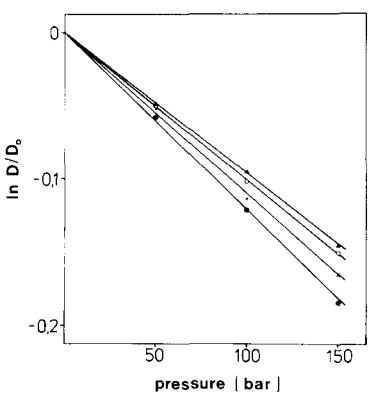

Fig. 2. Relative pressure induced change of the lateral diffusion coefficient of pyrene in pure and cholesterol containing dipalmitoylphosphatidylcholine membranes. The diffusion coefficient D, is given with respect to the diffusion coefficient D_0 at zero bar for each membrane preparation. Note the decreasing slope with increasing cholesterol content. Cholesterol amounts exceeding a mole fraction of $\rho = 0.09$ have no further effect. Mole fraction of cholesterol: ▲———▲, $\rho = 0$; ○———○, $\rho = 0.02$; ×———×, $\rho = 0.05$; ●———●, $\rho = 0.09$.

TABLE I

Relative change of the diffusion coefficient D at a given cholesterol content compared to pure dipalmitoylphosphatidylcholine membranes, where the diffusion is given by D_r. The pressure value to reach half reduction of the diffusion, $P_{1/2}$, as well as the pressure change of the transition temperature, dP/dT, are included. The dP/dT values were obtained from fluorescence polarization measurements using diphenylhexatriene as optical probes. Vesicles were prepared similar to the pyrene containing vesicles. We could not use the pyrene probes for the determination of dP/dT values due to the supression of the phase transition observed by the excimer technique at high cholesterol content (see Fig. 1).

mole fraction of cholesterol, ρ	D/D_r	$P_{1/2}$ (bar)	$\frac{dP}{dT}$ (bar/K)
0	1.0	575	34.9
0.02	0.91	627	38.5
0.03	–	–	39.5
0.05	0.82	686	41.1
0.07	–	–	42.2
0.09	0.63	720	43.5
0.13	–	–	46.1
0.17	0.53	720	50.0
0.23	0.44	725	55.5

one half of its original value by extrapolated 575 bars whereas in a membrane preparation containing a mole fraction of $\rho = 0.09$ cholesterol extrapolated 720 bars pressure have to be applied for a half-reduction of the diffusion coefficient (see Table I).

Earlier results on lateral diffusion gave evidence for the applicability of the free volume model at constant pressure. The aim of this paper is to introduce the pressure dependence of the lateral diffusion. The pressure decrease of the lateral diffusion in lipid bilayers is in excellent agreement with self diffusion measurements in liquid hydrocarbons. McCall et al. [15] reported the need of 530 bars in 2,2-dimethylhexane to reduce the diffusion by a factor of two. This value, predicted by the free volume model is well comparable to our value in dipalmitoylphosphatidylcholine bilayers.

Cholesterol is known to condense fluid bilayer membranes [16]. From our diffusion measurements we demonstrate that cholesterol membranes are less compressible. This could also be visualized from the pressure dependence of the lipid phase transition temperature, dP/dT, given in Table I. Our value for the pure dipalmitoylphosphati-

dylcholine vesicles of $dP/dT = 34.9$ bar/K is 20% smaller than values found by other authors [17,18]. The calculated value $dP/dT = 40$ bar/K using the DSC and density measurements of Nagle and Wilkinson [19] is in better agreement. Very recently Chong et al. [20] reported a value of 33 bar/K in dipalmitoylphosphatidylcholine membranes. The discrepancy may be due to different vesicle preparations. The authors in Refs. 17 and 18 used multilamellar preparations at low water content whereas we used sonified unilamellar vesicles. Despite the discrepancy we are able to observe a drastic increase in dP/dT to 55.5 bar/K at $\rho = 0.23$ cholesterol in dipalmitoylphosphatidylcholine membranes which clearly demonstrates the condensing effect of cholesterol.

At constant pressure the reduced average molecular area $\bar{a}_m$ and the reduced thermal expansion coefficient α [21] are thought to be responsible for the reduced lateral diffusion in the presence of cholesterol. The pressure effect on lipid diffusion and the need of a higher pressure increment in the presence of cholesterol to obtain a given reduction in the lateral diffusion can also be explained by the free volume model. From the change of the dP/dT values we derive qualitatively a measure for the change in the compressibility β. Thus we conclude that in cholesterol containing membranes the reduced compressibility β [21] has to be compensated by a higher pressure increment for a given decrease of the lateral diffusion as was already predicted from the diffusion equation given by the free volume model.

To summarize: earlier results on lateral diffusion at constant atmospheric pressure [11] gave evidence for the applicability of the free volume model. We have now extended the diffusion equation by the pressure term (Eqn. 1). The observed pressure dependence of the lateral diffusion in pure DPPC and mixed DPPC-cholesterol membranes are again satisfactorily described by this model giving further evidence for its validity. However, a concrete distinction between this model and the hydrodynamic Saffman-Delbrück model is not yet possible from our experiments.

Such a distinction could be obtained from the molecular weight dependence of the diffusing species. Photobleaching experiments will be presented in another paper [22] that compares the lateral diffusion of lipids and proteins. Unfortunately the error bars in the diffusion values are still too high to distinguish between a $M^{-1/2}$ mass dependence (M is the molecular weight of the diffusing molecule) and the rather logarithmic dependence in the hydrodynamic model. It appears, however, that the Saffman-Delbrück model is more appropriate to describe the lateral diffusion of integral macromolecules whereas the free volume model describes the diffusion of small solutes.

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