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Minor effects of bulk viscosity on lipid translational diffusion measured by the excimer formation technique

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Abstract. We have investigated the effect of bulk viscosity on lipid translational diffusion using the excimer formation technique. In contrast to a study by Vaz et al. (1987), performed with the fluorescence recovery after photobleaching technique, we observed only a minor decrease of less than a factor of two for pyrene labelled phosphatidylcholine in glycerinated phosphatidylcholine bilayer membranes compared to an aqueous dispersion. Even the diffusion of pyrene labelled gangliosides with an oligosaccharide headgroup that protrudes from the membrane surface is not strongly restricted by the increased bulk viscosity. We conclude that the viscosity of the fluid bounding the lipid bilayers is of minor importance for the diffusion of membrane lipids.

Key words: Translational diffusion, excimer formation, glycerol, interdigitated phase

Introduction

The translational diffusion of lipids and proteins in bilayer membranes has been investigated by a large number of techniques (for a review see Clegg and Vaz 1985). Two theoretical models have been used to explain the results. The hydrodynamic model for diffusion in viscous fluid sheets (Saffman and Delbrück 1975; Saffman 1976) adequately describes the diffusion of proteins (Vaz et al. 1982) whereas lipid diffusion has been successfully described by the free volume model of Cohen and Turnbull (1959) developed for diffusion processes in liquids. Galla et al. (1979) applied this model for diffusion in lipid bilayers measured by the excimer formation technique (Galla and Sackmann 1974; Galla and Hartmann 1980). Among other experimental evidence it was concluded from the chain length dependence of lateral diffusion that lipid diffusion is better described by the free volume model (Müller and Galla 1987; Vaz and Hallmann 1983; Vaz et al. 1985). Further evidence for this model came from diffusion measurements in lipid monolayers at the air-water interface (Peters and Beck 1983).

Until recently, although described theoretically (Hughes et al. 1981, 1982) and despite the great importance of membrane surface structures for biological membranes, only the viscosity of the lipid bilayers has been considered to influence translational diffusion in fluid membranes. Vaz et al. (1987) were the first to show, using the fluorescence recovery after photobleaching technique, that translational diffusion of a fluorescence labelled lipid derivative is strongly influenced by the viscosity of the bulk phase. For a membrane spanning lipid in glycerinated L_α-phase multibilayers made of 1-palmitoyl-2-oleylphosphatidylcholine they observed a drastic decrease of the translational diffusion coefficient. At $T = 40\,°C$ D was reduced from 5×10^{-8} cm^2/s for membranes in water to 2.4×10^{-9} cm^2/s for membranes dispersed in pure glycerol. The same is true for $T = 10\,°C$ where D was reduced from 2×10^{-8} cm^2/s to 3×10^{-9} cm^2/s. This effect can not be adequately described by the existing models. However, these results are not supported by the experiments presented in this paper. With the excimer formation technique we only observed a decrease of the lateral diffusion coefficient by a factor of two in dipalmitoyl- and in distearoylphosphatidylcholine membranes measured at 10 °C above the corresponding lipid phase transition temperature in hy-

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Abbreviations: DPPC: 1-2 dipalmitoyl-sn-glycero-3-phosphocholine; DSPC: 1-2 distearoyl-sn-glycero-3-phosphocholine; PyPC: 1-acyl-2-[10(-1-pyrene)decanoyl]-sn-glycero-3-phosphocholine; PyG_{M1}: N-12-(1-pyrene)dodecanoyl-lyso G_{M1}; PyG_{M2}: N-12-(1-pyrene)dodecanoyl-lyso G_{M2}; PyG_{M3}: N-12-(1-pyrene) dodecanoyl-lyso G_{M3}; I_M: fluorescence intensity of the monomeric pyrene probe; I_D: fluorescence intensity of the excimer

drated and in glycerinated membranes. Further experiments using other techniques or probes seem to be necessary to verify these conflicting results and thus to get a better understanding of the mechanism of translational diffusion in lipid bilayer membranes.

Materials and methods

Phospholipids were obtained from Fluka (Neu Ulm, FRG) and used without further purification. Pyrene labelled phosphatidylcholine was synthesized in our own laboratory by J. Becker as described elsewhere (Galla and Hartmann 1981). Pyrene labelled gangliosides (PyG_{M1}, PyG_{M2} and PyG_{M3}) were synthesized according to Schwarzmann and Sandhoff (1987).

DPPC and DSPC vesicles containing pyrene-labelled phosphatidylcholine or pyrene-labelled gangliosides as excimer forming probes were prepared by moderate sonication. In short: lipids were dissolved in chloroform/methanol (1:1 v/v). The solvent was removed by a stream of nitrogen at a temperature 5 °C above the phase transition temperature of the corresponding lipid and by subsequent evaporation in a vacuum oven for 2 h. Water or glycerol (99% water free) was added to make up a final lipid concentration of 1 mg/ml. Vesicles in water were obtained by 3 min sonication (Branson sonifier B15, microtip, 20 W), whereas vesicles in glycerol needed to be sonicated for 10 min. Samples in pure glycerol were diluted with water to the desired glycerol/water ratio and were equilibrated for several hours at 21 °C before fluorescence measurement. The excimer/monomer fluorescence intensity ratio was not affected by the dilution.

Fluorescence spectra were recorded with a Perkin Elmer MPF3 spectrometer equipped with controlled temperature regulation. Fluorescence intensities were measured at 475 nm for the excimer (I_D) and at 396 nm for the monomer intensity (I_M).

The diffusion coefficients can be determined from the fluorescence intensities of the excimer, I_D, and the monomer, I_M of pyrene derivatives incorporated into the membrane. The intensity ratio I_D/I_M is a measure of the diffusion controlled excimer formation of the pyrene derivatives (Galla and Sackmann 1974). The jump frequency, ν_j, in a lipid matrix is directly related to the intensity ratio by $\nu_j \sim \text{const} \cdot I_D/(I_M \cdot \tau_{0D})$ where τ_{0D} is the excimer lifetime which has to be measured separately. The coefficient of 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 neighbouring lipid molecules (0.8 nm). In this paper translational diffusion is characterized by the I_D/I_M-ratio. For further details see Galla and Hartmann (1980) and Galla et al. (1979).

Results and discussion

We investigated the effect of glycerol on the excimer formation of pyrene-labelled phosphatidylcholine (PyPC) and of pyrene-labelled gangliosides PyG_{M1}, PyG_{M2} and PyG_{M3} in phosphatidylcholine membranes. Figure 1 shows the temperature dependence of the excimer to monomer fluorescence intensity ratio I_D/I_M of PyPC in aqueous and in glycerinated dipalmitoylphosphatidylcholine bilayers. In the aqueous medium we obtained a typical phase transition curve with a pretransition at $T_p = 33$ °C and the main transition at $T_m = 41$ °C. In the presence of increasing amounts of glycerol the pretransition deminishes and disappeared completely at 80% (v/v) glycerol/water corresponding to a mole fraction of $x_G = 0.50$. We conclude that even in pure glycerol, lipid layers are formed that exhibit a phase transition at the same temperature where the hydrated bilayer undergoes the gel to liquid-crystalline phase transition. This is in agreement with earlier experiments using x-ray diffraction and differential scanning calorimetry (McDaniel et al. 1983). These authors reported the formation of a fully interdigitated gel phase at a glycerol content exceeding a glycerol molar ratio of $x_G = 0.5$. Fully interpenetrated hydrocarbon chains are oriented perpendicular to the plane of the membrane and from the $L_{\beta I}$-phase. Thus the appearance of a pretransition from the $L_{\beta'}$- to the $P_{\beta'}$-phase is excluded.

The shape of the phase transition curves in excess glycerol is also explainable by the assumption of an interdigitated gel phase. In the light of two recent papers (Hresko et al. 1986; Ollmann et al. 1987) the shape of the phase transition curve obtained by the excimer technique is a measure for the immiscibility of the probe within the gel phase. A comparatively sharp peak of the I_D/I_M ratio with temperature was observed in 90% or in pure glycerol between 35° and 43 °C which is typical for a probe with a non-ideal mixing behavior. Such behavior could be expected for the bulky pyrene derivative causing a reduced solubility or clustering in the interdigitated phase.

Our main point of interest was however the temperature range of the fluid L_α-phase. There the I_D/I_M ratio is a direct measure of the lateral diffusion coefficient of the probe molecule (see materials and methods). It is clear from Fig. 1 that glycerination only slightly reduces the I_D/I_M ratio. Figure 2 shows the change in the I_D/I_M ratio with increasing molar fraction of glycerol for PyPC in DPPC and DSPC membranes at a temperature 10 °C above the corresponding lipid phase transition temperature. Depending on lipid chain length the excimer yield is higher in DSPC membranes (Müller and Galla 1987). The major result is that the I_D/I_M value is only reduced by a factor of two if water is substituted by pure glycerol. The drastic

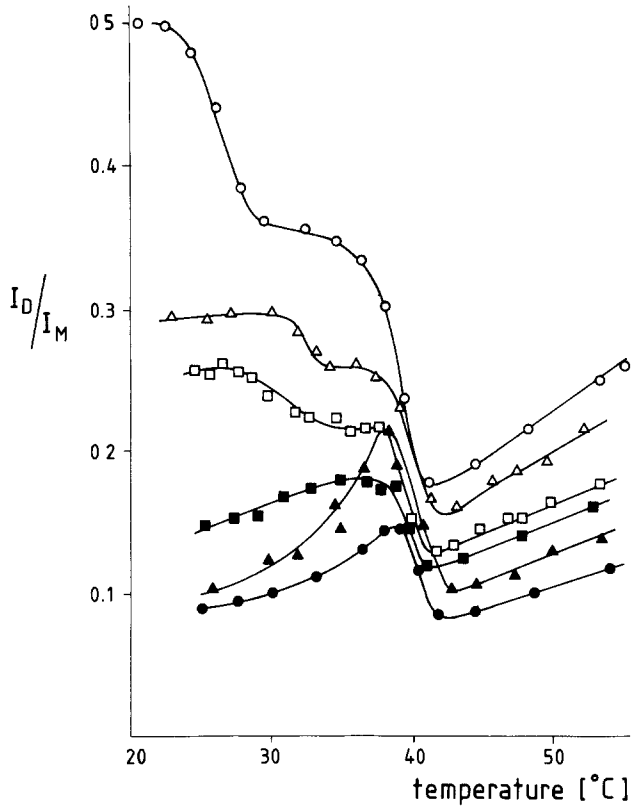

Fig. 1. Phase transition curves of dipalmitoyl phosphatidylcholine vesicles in water, in water/glycerol in a given mole fraction x_G and in pure glycerol. Pyrene labelled phosphatidylcholine (2 mol%) was used as probe. $x_G = 0$ (o-o-o), $x_G = 0.14$ (Δ-Δ-Δ), $x_G = 0.37$ (□-□-□), $x_G = 0.5$ (■-■-■), $x_G = 0.69$ (▲-▲-▲), $x_G = 1$ (●-●-●)

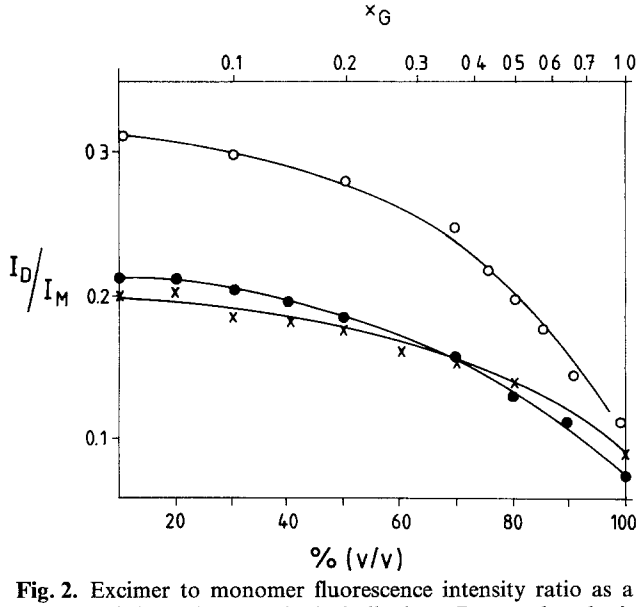

Fig. 2. Excimer to monomer fluorescence intensity ratio as a function of glycerol content in the bulk phase. Pyrene phosphatidylcholine was used as probe molecule in distearoylphosphatidylcholine (o-o-o) at T = 65 °C and in dipalmitoylphosphatidylcholine (●-●-●) at T = 50 °C. Pyrene labelled gangliosides PyG_{M1}, PyG_{M2} and PyG_{M3} in DPPC membranes had identical values within the error of determination (×-×-×)

reduction of the diffusion coefficient by at least an order of magnitude reported by Vaz et al. (1987) could not be observed. Obviously the choline headgroup of the diffusing probe is not drastically constrained by the viscous bulk phase. We therefore used pyrene labelled gangliosides with an oligosaccharide head group protruding out of the membrane and into the bounding fluid. Again a reduction of less than a factor of two was measured in glycerinated membranes although the viscosity of glycerol is 250 times larger than that of water (Landolt-Börnstein 1969). Taking into account the free volume model (Galla et al. 1979) and considering the translational friction coefficient introduced by Vaz et al. (1985) we come to the conclusion that the translational frictional drag upon a membrane particle is mainly the drag of the lipid bilayer, whereas the drag in the membrane bounding fluid is of minor importance.

At present we have no explanation for the conflicting results obtained by the excimer and the photobleaching technique. Therefore this paper must not be construed as an expression of criticism but hopefully stimulates further clarifying experiments. Although not convincing it has to be considered that different probes were used and that the recovery after photobleaching technique measures long distance diffusion whereas the excimer technique measures short distance diffusion. Moreover we used sonified lipid dispersions whereas Vaz et al. (1987) used multilamellar lipid sheets which might influence the diffusion. Finally we need to ask whether a fluid phase immiscibility of the probe at high glycerol keeps I_D/I_M high and therefore might mask a slow lipid diffusion. However the temperature dependence of the I_D/I_M ratio in the L_α-phase (Fig. 1) and also the increase of I_D/I_M with probe concentration at a given temperature is linear. A fluid phase immiscibility was not observed.

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