Translational diffusion in phospholipid bilayer membranes

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(Received 15 August 1997; revised manuscript received 24 November 1997)

The translational diffusion in artificial phospholipid bilayers has attracted much interest in the past. This process is widely accepted to be of importance for the diffusion of biological membrane components. The lipid bilayer is known as the permeability barrier in biological membranes. So far the diffusion of the membrane components has been determined indirectly by sophisticated methods, e.g., by the help of fluorescent probes. We present here experimental data on the orientational relaxation of the lipid headgroup in alternating electric fields. The dipole relaxation time measured by high-frequency spectroscopy is shown to be related to the self-diffusion coefficient. We have determined the electric dipole relaxation of two phospholipids and of their mixture. The dielectric relaxation time and thus the diffusion coefficient was measured as a function of temperature in both the liquid and the gel crystalline phase. The mixture even allows one to study the relaxation and the diffusion behavior in a state where both gel and liquid phases coexist. The experimental study has revealed the potential of high-frequency dielectric spectroscopy to determine the diffusion coefficient and its thermal activation energy in phospholipid membranes where the dipolar relaxation of the headgroup is associated with a site change. [S1063-651X(98)00804-6]

PACS number(s): 87.22.Bt, 77.22.Gm, 66.10.Cb, 64.70.Md

Phospholipids, of which biological membranes are built, are amphiphilic molecules with a strong polar headgroup. The membrane consists of a bilayer structure induced by the water molecules at its surface as illustrated in Fig. 1 [1,2]. The zwitterionic dipolar headgroup is assumed to rotate around the P—O bond, or less probably around the O—C bond as indicated in Fig. 1 [3]. However, there have been indications from NMR experiments that the headgroup rotation is performed by an axial rotation of the entire molecule around the bilayer normal [4]. The frequency of the rotational relaxation was observed in the MHz region [5]. The headgroup is localized by interlamellar and intralamellar hydrogen bonds of the water molecules near the bilayer's surface. Essentially these bonds induce the lateral organization of the bilayer structure [1]. The investigated samples are made from crystalline 1,2-dimyristoyl-phosphatidyl-choline and from 1,2-distearoyl-phosphatidyl-choline (L-α-DMPC and L-α-DSPC, Nattermann GmbH/Köln). Both DMPC and DSPC have the same headgroup but different alkyl chain lengths, i.e., 14 and 18 CH_2 groups, respectively. The main phase transition takes place in DMPC at 23 °C and in DSPC at 54 °C. The samples were prepared with excess water (50% of weight) and homogenized for 24 h at 60 °C. The samples form multilamellar vesicles as illustrated in Fig. 1.

The relaxation frequency of the headgroup is obtained from the dielectric response of the samples, i.e., from the complex dielectric function (DF):

$$\epsilon(\nu,T)=\epsilon_1(\nu,T)-i\,\epsilon_2(\nu,T), \tag{1}$$

where ν is the frequency and T is the temperature. The DF's real and imaginary components describe the polarizability and the dissipation of the material, respectively. The experimental setup used to measure $\epsilon(\nu,T)$ is described elsewhere [6]. In Fig. 2 experimental results of the dissipative component ϵ_2 as a function of frequency are presented for both the liquid and the gel phases. At low frequencies the dielectric response is dominated by dc conductivity due to ionic impurities. The measurements can be described by the Havriliak-Negami equation for unsymmetric broadened relaxation functions [7]:

$$\epsilon^{\star}(\omega)=\frac{\epsilon_s-\epsilon_\infty}{[1+(i\omega\tau_D)^{\alpha}]^{\beta}}+\epsilon_\infty-i\,\frac{\sigma_{\mathrm{dc}}}{\epsilon_0\omega}, \tag{2}$$

where $\omega=2\pi\nu$ denotes the angular frequency and τ_D the relaxation time. α and β are a measure for the broadening ($\beta=1$ for symmetric broadening). For a Debye process with a single relaxation time ($\alpha=\beta=1$) the half-width of the relaxation peak in ϵ_2 is 1.14 frequency decades. In our case half-widths of 1.3–1.8 frequency decades are observed. The asymmetry of the relaxation peak is due to an additional contribution of bound water, which has its maximum response at higher frequencies [8,9]. The broadening corresponds to a distribution of relaxation times, e.g., due to different local structures. The Debye frequency at which the maximum of ϵ_2 is observed is a good measure for the average dipole relaxation time [10]:

$$\nu_D=\frac{1}{2\pi\tau_D}. \tag{3}$$

Here ν_D corresponds to the relaxation frequency of the headgroups [5]. Cooling the samples the Debye frequency shifts to lower frequencies. In both systems the frequency drops by about one order of magnitude to lower values at the liquid to gel phase transition. Obviously, the dipole relaxation and thus the diffusion is much slower in the solid gel phase than in the liquid phase as a consequence of the higher order. However, in the mixture having a two-phase coexistence of gel and liquid phase [11], the relaxation depends quasicontinuously on temperature.

An Arrhenius plot of the temperature dependence of the Debye frequency yields the activation energy for the different crystalline phases (linear regions above and below the

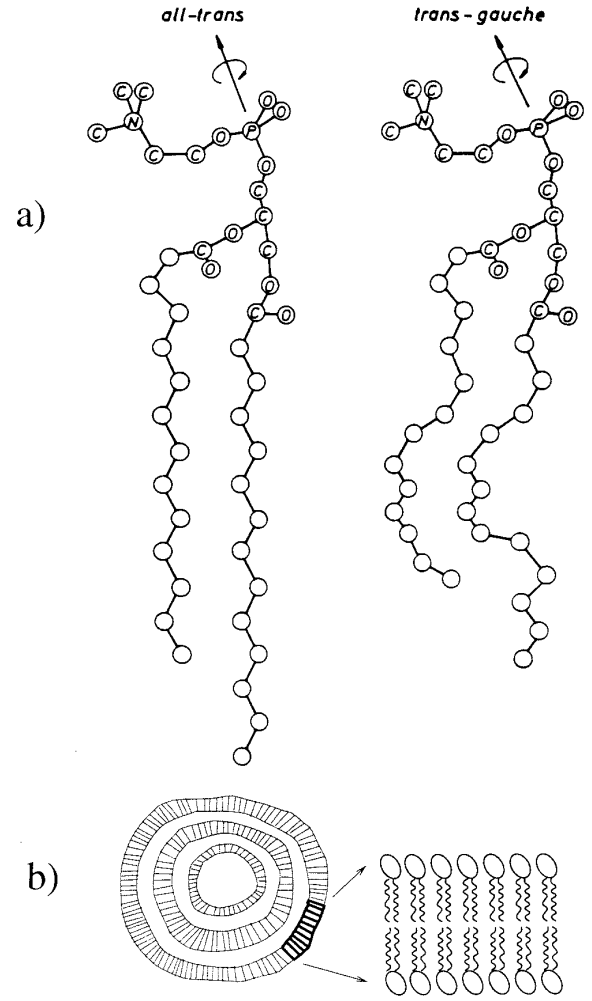

FIG. 1. (a) Phosphocholin headgroup and alkyl chains of the phospholipid DMPC. Left hand side shows the molecule in the all-trans configuration of the gel phase, whereas on the right hand side the molecule shows some gauche configurations representing the liquid phase. (b) A sketch of the bilayer structure and the vesicles studied, in which the individual lipid bilayers are separated by water layers.

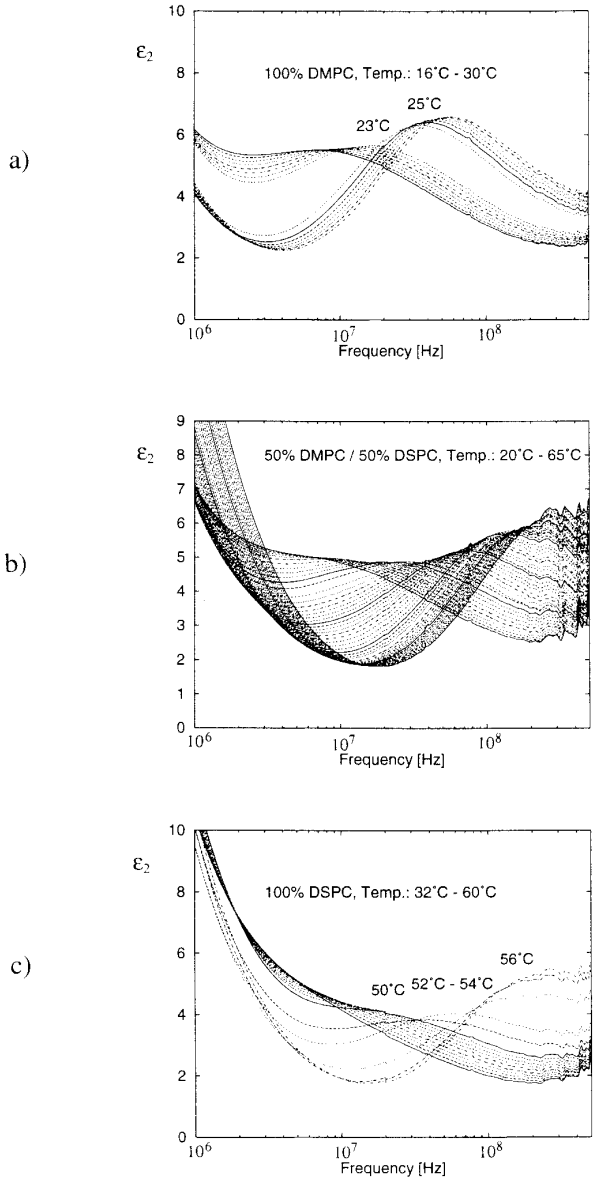

FIG. 2. ϵ_2 vs frequency. (a) for DMPC, (b) for a 50/50 DMPC-DSPC mixture, and (c) for DSPC.

phase transitions, see Fig. 3). The Debye relaxation frequency depends on the temperature according to the relationship [10,12]

$$\nu_D = \nu_0 \exp\left(\frac{-\Delta H}{k_B T}\right). \tag{4}$$

ν_0 is in first order approximation a constant [10] and k_B is the Boltzmann constant. ΔH is the enthalpy which corresponds to the activation energy obtained from the Arrhenius plot. The phase transition temperature depends on the length of the alkyl chain. However, in temperature ranges where the molecules are in the liquid or in the gel phase, respectively, they display nearly the same relaxation frequency. Since they have the same headgroups, obviously their rotation essentially determines ν_D. The DSPC bilayers show an additional intermediate phase in the displayed temperature range. The transition from the gel to the liquid phase is remarkably pronounced.

The activation energies of both molecules are $\approx$480 meV in the liquid phase and $\approx$1180 meV in the gel phase. Qualitatively this result reveals that in the solid state about four hydrogen bonds, whereas in the liquid phase only two bonds, have to be broken in order to rotate and move the phospholipid molecule (the activation energy due to hydrogen bonds in water is 213 meV [12]). The bilayer behaves similarly to water, the latter having an activation energy nearly tripled at the transition from water to ice [12]. We want to point out that the activation energy of the Debye relaxation process has a value near to that found for the diffusion of a lipid probe molecule (see Fig. 4). In fact we shall show that under certain conditions the knowledge of the Debye relaxation time provides an estimate of the translational diffusion behavior [10,12]. As mentioned above, hydrogen bonds of the headgroup induce the lateral organization of the bilayer structure (between the alkyl chains there are only weak van der Waals interactions). A translation is only possible in the presence of structural defects, i.e., vacancies in the two-dimensional (2D) liquid crystalline bilayer (see Fig. 5). Due to steric reasons (the inclination of the large headgroup, see

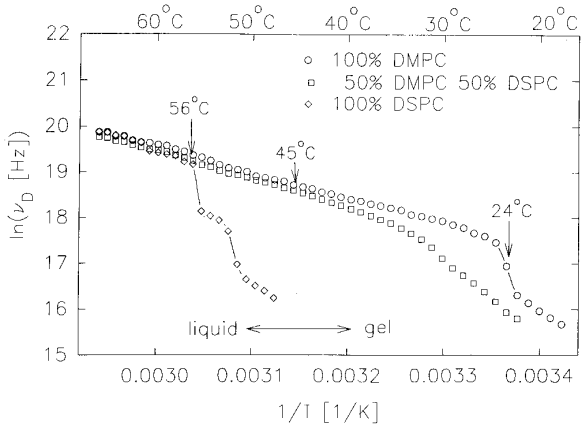

FIG. 3. Arrhenius plot of the headgroup relaxation frequency for DMPC, DSPC, and a 50/50 mixture. The gel phase has an activation energy of ≈1180 meV and the liquid crystalline phase ≈480 meV.

Fig. 1 [1]) the molecule has to rotate in order to change its position. As in the case of a rolling cylinder the translation implies a rotational operation. For example, this process of diffusion is well established in water [12]. The dipole relaxation time of the headgroup presents a measure for the rotational diffusion and consequently for the lateral movement.

The self-diffusion coefficient depends on the mean free path l and the thermal velocity v_{th}. For the lateral movement inside the membrane the two-dimensional case is to be considered:

$$D_{\mathrm{lat}}=\tfrac{1}{2}\,v_{\mathrm{th}}l. \tag{5}$$

The mean free path corresponds to a site change of the molecule in the bilayer lattice (see Fig. 5):

$$l=2a, \tag{6}$$

where a is the molecule's mean headgroup radius (we use $a=0.4$ nm [1,2]). The thermal velocity is given by

$$v_{\mathrm{th}}=\frac{l}{\tau_{\mathrm{trans}}}, \tag{7}$$

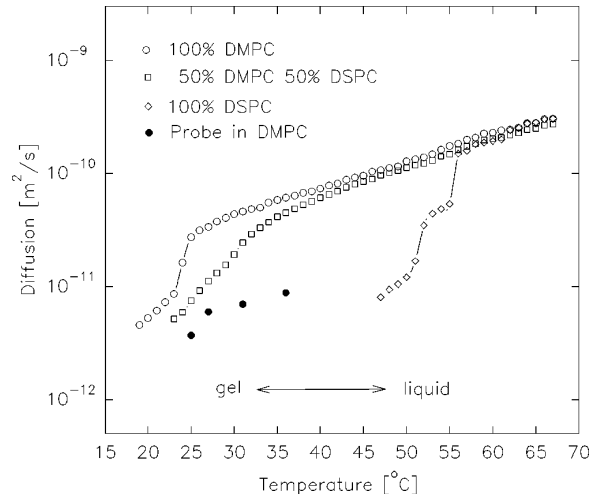

FIG. 4. Average diffusion coefficient obtained from the Debye relaxation time for DMPC and DSPC with Eq. (11) and $a=0.4$ nm [1,2]. The filled circles represent experimental values obtained with fluorescent probes in DMPC [13].

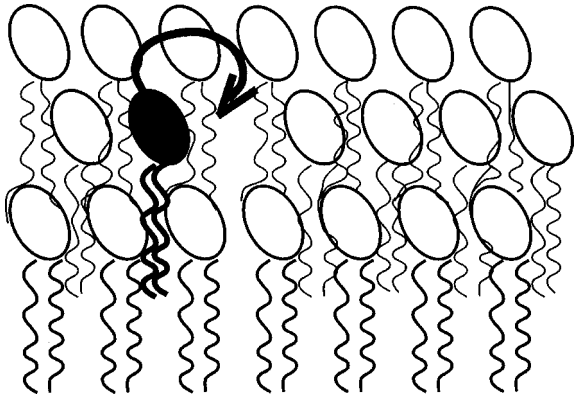

FIG. 5. Sketch of dipole relaxation and molecular site change in the bilayer structure.

where the scattering time τ_{trans} corresponds to the average time for a site change. Equations (6) and (7) yield

$$D_{\mathrm{lat}}=\frac{a^2}{\tau_{\mathrm{trans}}}. \tag{8}$$

In order to correlate the translational diffusion process to the rotational relaxation measured by dielectric spectroscopy, a relation between τ_{trans} and τ_D has to be established. Since we are considering the liquid phase of the membrane, we might assume that each degree of freedom takes up the same thermal energy, i.e., the average rotational energy E_{rot} equals the average translational energy E_{trans}. However, in our *fluid lattice* a site change is only possible in the presence of a vacancy. Otherwise it is blocked and thus on average $E_{\mathrm{trans}}\leqslant E_{\mathrm{rot}}$:

$$\tfrac{1}{2}\,mv_{\mathrm{th}}^2\leqslant\tfrac{1}{2}\,J\omega^2, \tag{9}$$

where m denotes the mass of the molecule, ω the angular frequency of the rotation, and J the moment of inertia. ω is given by the relaxation time of the headgroup dipole, i.e., $\omega=1/\tau_D$. For a homogeneous cylinder rotating around its main axis $J=\frac{1}{2}ma^2$ holds and thus

$$v_{\mathrm{th}}\leqslant\frac{a}{\sqrt{2}\,\tau_D}. \tag{10}$$

A comparison with Eq. (7) yields $\tau_{\mathrm{trans}}\geqslant\sqrt{8}\,\tau_D$. This may be interpreted as a superposition of a continuous translation and rotation, or as a sequence of two processes with different probabilities p_i: a rotation associated with a site change ($p_1\leqslant 1/\sqrt{8}$) and a rotation on a given lattice site ($p_2\geqslant 1-1/\sqrt{8}$). The latter interpretation seems to be more adequate since we consider a *fluid lattice*. In such systems the knowledge of the Debye relaxation provides an estimate of the 2D lateral self-diffusion coefficient of cylindrical molecules [Eqs. (5), (6), and (10)]:

$$D_{\mathrm{lat}}\leqslant\frac{a^2}{\sqrt{2}\,\tau_D}. \tag{11}$$

Note that for three-dimensional systems $D_{\mathrm{trans}}=\frac{1}{3}v_{\mathrm{th}}l$. In the case of diffusing spheres $J=\frac{2}{5}m(\omega a)^2$ and a similar calculation yields

$$D_{\mathrm{trans}}\leqslant\frac{2}{3}\sqrt{\frac{2}{5}}\frac{a^2}{\tau_D}. \tag{12}$$

Apart from the factor $\sqrt{2/5}\simeq 0.63$ this result is obtained relating the Debye and the Stokes-Einstein equations [10,12]:

$$D_{\text{trans}} = \frac{k_B T}{6\pi\eta a}, \tag{13}$$

$$\tau_D = \frac{8\pi\eta a^3}{2k_B T}, \tag{14}$$

and thus

$$D_{\text{trans}} = \frac{2}{3}\frac{a^2}{\tau_D}. \tag{15}$$

Equation (13) describes the translational diffusion of a hard sphere in a liquid of viscosity η, while Eq. (14) has been derived for the rotation of a dipolar sphere in a nonpolar liquid. Thus Eq. (15) is not *a priori* expected to describe also liquids the molecules of which are associated by hydrogen bonds [10,12]. The interaction with the surrounding polar molecules depends on details of the movement. This might be taken into account in Eqs. (13) and (14) assuming different hydrodynamic radii or local viscosities and would result in a modified Eq. (15). In contrast to the theoretical restriction, however, diffusion as well as NMR studies have confirmed the above relationship between D_{trans} and τ_D in the case of water [12]. Our plausible derivation shows that Eqs. (11) and (12) (for spheres in 3D), or Eq. (15) (for cylinders in 2D) hold as long as the dipolar relaxation (rotation) is associated with a translation, so that the interactions for both processes are equal.

Due to the above mentioned distribution of relaxation times our τ_D values and thus the self-diffusion coefficients are averaged quantities. The self-diffusion coefficient of DMPC and DSPC as a function of temperature is presented in Fig. 4. For comparison we show four values of the diffusion coefficient obtained with a fluorescent probe technique (probe molecule NBD-DMPE [N-(7-nitro-2,1,3-benzoxadiazol-4-yl)-dimyristoyl-phosphatidyl-ethanolamine]) [13]. The shapes of the curves are similar and the values are of the same order of magnitude. They differ by about a factor of 7. Three reasons can account for this effect: (i) It has already been shown that in fluorescent techniques the size of the probe molecule has a significant influence on the apparent D values [13]. Similarly, lower diffusion values may be caused by the special interaction of the probe molecule with the phospholipid membrane molecules. (ii) We have used the geometrical radius $a = 0.4$ nm to calculate the thermal velocity [Eq. (10)] although the hydrodynamic radius may be smaller [14]. (iii) The values obtained from dielectric spectroscopy using Eq. (11) are an upper limit for D_{lat}. As we have shown, the probability for a translation may be reduced since in the fluid lattice a site change is only possible in the presence of a vacancy. Therefore a more precise microscopic model is required to calculate the exact ratio $\tau_{\text{trans}}/\tau_D$. The data shown in Fig. 4 reveal two interesting features: in the liquid phase both DMPC and DSPC have the same diffusion coefficient ($T \geqslant 55\,^\circ\text{C}$) whereas the 50/50 mixture has a smaller diffusion coefficient than the pure systems. This effect may be due to the higher disorder in the mixture.

We have shown that the translational motion of a phospholipid molecule is associated with a rotation of the headgroup. Thus dipolar relaxation time and diffusion coefficient are related [see Eq. (11)]. The dipole relaxation measurements presented have given an average diffusion coefficient for the gel as well as for the liquid phases, even for the binary mixture with a temperature region of coexistance of the gel and liquid phase ($27\,^\circ\text{C} \leqslant T \leqslant 55\,^\circ\text{C}$). An abrupt change of the relaxation time by about one order of magnitude at the phase transition is observed. In the regime of the coexistence of the two phases, however, which may be relevant for a more complex biological membrane, the relaxation and thus the diffusion increases quasicontinuously with temperature. In addition to the diffusion properties, the measurement of the temperature dependence of the molecular dipole relaxation yields the activation energy of the diffusion process in the different phase states.

[1] R. H. Pearson and I. Pascher, Nature (London) **281**, 499 (1979).

[2] E. Sackmann, Ber. Bunsenges. Phys. Chem. **82**, 891 (1978).

[3] L. Trahms and W. Klabe, Mol. Cryst. Liq. Cryst. **123**, 333 (1985).

[4] M. Fuson and J. Prestegard, Biochemistry **22**, 1311 (1983).

[5] G. Nimtz, A. Enders, and B. Binggeli, Ber. Bunsenges. Phys. Chem. **89**, 842 (1985).

[6] R. Pelster, IEEE Trans. Microwave Theory Tech. **43**, 1494 (1995).

[7] S. Havriliak and S. Negami, J. Polym. Sci. Part C **14**, 99 (1966).

[8] A. Enders and G. Nimtz, Ber. Bunsenges. Phys. Chem. **88**, 512 (1984).

[9] B. Klösgen, C. Reichle, S. Kohlsmann, and D. Kramer, Biophys. J. **71**, 3251 (1996).

[10] H. Fröhlich, *Theory of Dielectrics* (Clarendon Press, Oxford, 1949).

[11] W. Knoll, K. Ibel, and E. Sackmann, Biochemistry **20**, 6379 (1981).

[12] F. Franks, *Water* (The Royal Society of Chemistry, London, 1983).

[13] W. Vaz, M. Criado, V. Madeira, G. Schoellmann, and Th. Jovin, Biochemistry **21**, 5608 (1982); W. Vaz, E. Melo, and Th. Thomson, Biophys. J. **56**, 869 (1989).

[14] F. Fujara, B. Geil, H. Sillescu, and G. Fleischer, Z. Phys. B **88**, 195 (1992).