Correlation and dynamics of acyl-chain isomerization rotation in di-myristoyl-phosphatidylcholine bilayers

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The dielectric behavior of hydrated di-myristoyl-phosphatidylcholine (DMPC) bilayers was investigated as a function of temperature at frequencies from 4 to 12 GHz. A steplike increase of the dielectric constant occurs at the chain melting phase transition. It is related to a dielectric dispersion due to the onset of the acyl-chain isomerization rotation. The measured dielectric response, with its unexpectedly high values, is interpreted as a cooperative rotational motion within large chain clusters. A lower limit for the cluster size of at least 250 CH_2—CH_2 bonds with correlated rotation is deduced.

I. INTRODUCTION

Di-myristoyl-phosphatidylcholine (DMPC) consists of two saturated C_{14} carbon acids [myristic, or tetradecanoic, acid; $CH_3(CH_2)_{12}COOH$] connected to a hydrophilic head group of the lecithin type. Dissolved in water, DMPC forms multilamellar aggregates consisting of DMPC bilayers and intermediate (bound) water. A main feature of the physical behavior of this and related systems is the so-called gel-fluid phase transition. It is characterized by a conformational change of the acyl chains from an ordered all-trans ground state to a disordered fluidlike phase (chain melting transition), in which the chains undergo rapid isomerization rotations between excited *gauche* conformations. This behavior is interesting with regard to the physical state of biological membranes (whose lipid bilayer matrix has a similar structure)[1,2] and the process of polymer melting.[3] Quite recently, new theories have been developed to achieve a better understanding of both subjects.[4-8] In this Brief Report we report on dielectric data which elucidate several properties of the chain isomerization rotation as the correlation of excited conformations, the relaxation time, and the role of hydration.

II. EXPERIMENTAL

Samples with different water content (see Table I) and total weights of about 200 mg were prepared from crystalline L-α-DMPC (99%, Sigma), sealed in a polyvinyl chloride (PVC) sandwich structure and homogenized 20 K above the main phase transition temperature for 10 h. The dielectric properties of the samples were measured in two microwave bridges between 4–6 and 8–12 GHz, respectively. The experimental setup and the numerical evaluation of the complex dielectric constant from transmission data are reported elsewhere.[9] The data were continuously recorded as a function of temperature at heating and cooling rates of 0.2 K/min.

III. RESULTS AND DISCUSSION

The complex dielectric constant $\epsilon = \epsilon_1 + i\epsilon_2$ of two samples having different rates of hydration is represented in Fig. 1. The phase transition is marked in mixture (1) by a positive step spread over a temperature interval of 10 K. In sample (6) there is a negative step over an interval of 3 K,

TABLE I. Dielectric and thermodynamic properties of the chain melting phase transition in DMPC bilayers (heating runs).

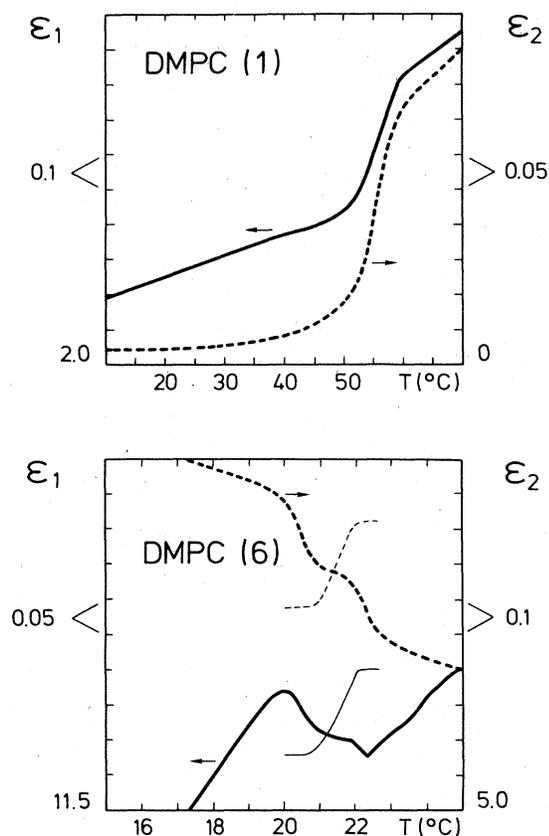| Sample	$\dfrac{H_2O\text{-mol}}{DMPC\text{-mol}}$	Measured frequency f_M (GHz)	$\Delta\epsilon_1$	$\Delta\epsilon_2$	Relaxation frequency f_D (GHz)	$(\Delta\epsilon_s)_s$ of sample	$(\Delta\epsilon_s)_c$ of chains	Transition temp. (°C)	Transition width (K)
(1)	2	4.5	0.26	0.48	2.4	1.1	1.2	60	10
		8.3	0.35	0.30	9.7	0.6	0.6		
		9.1	0.35	0.41	7.8	0.8	0.9		
(2)	5	9.1	0.20	0.30	6.0	0.6	0.7	55	10
		10.1	0.17	0.30	5.7	0.7	0.8		
(3)	13	9.1	Not resolvable		$\cdots$	$\cdots$	$\cdots$	$\cdots$	$\cdots$
(4)	20	8.3	$\cdots$	0.06	$\cdots$	$\cdots$	$\cdots$	22	4
(5)	32	9.1	$\cdots$	0.12	$\cdots$	$\cdots$	$\cdots$	22	2.5
(6)	47	4.5	0.20	0.17	5.3	0.3	0.8	22	1
		8.3	0.12	0.24	4.2	0.6	1.3		
		9.1	0.20	0.17	10.7	0.3	0.8		
		10.1	0.16	0.25	6.5	0.6	1.2		

FIG. 1. Complex dielectric constant as a function of temperature for mixtures (1) and (6) at a frequency of 8.2 GHz. The contribution of the chain melting transition in mixture (6) is illustrated by positive steps (thin lines). The data are recorded in the heating run.

which is, however, superposed by a positive structure with ~ 1 K width, as demonstrated in Fig. 1 for both ϵ_1 and ϵ_2. The main contribution of the total complex dielectric constant was found to be the dielectric relaxation of the water molecules, which are more or less bound as interlamellar water.[10] The negative steps in samples with high water content (as, e.g., mixture 6) were related to an additional binding of water molecules with increasing temperature near the chain melting transition. The negative signal corresponds to one broad step. This result followed from the dependence of the dielectric response on water concentration near the phase transition.[10] Between heating and cooling runs the negative and positive steps are shifted in opposite directions revealing the superposition of two single steps of opposite sign and different temperature interval in the dielectric response. A further contribution to the dielectric constant of the water/DMPC samples is the polarizability of the DMPC molecules.

In this Brief Report we want to focus on the positive structures observed in the dielectric response at the phase transition. These steps are nearly of equal magnitude both in $\Delta\epsilon_1$ and $\Delta\epsilon_2$ (see Table I). According to the Debye equations,[11] they may either be caused by a drastic increase of the dipole relaxation frequency f_D of any of the water molecules in the samples to a value near the measuring mi-

crowave frequency, or a new dielectric relaxator is created, namely, the chain melting motion. The first possibility can be excluded, because, in this case, the temperature-dependent dielectric response of these water molecules must show a maximum in ϵ_2 above the phase transition. Particularly in mixture (1) such an effect would produce a pronounced maximum in the ϵ_2 response, because there is only one type of water relaxator due to the low water concentration, namely, two water molecules per lipid molecule. But this is not the case; on the contrary, both ϵ_1 and ϵ_2 increase continuously with temperature. In mixtures with higher water concentrations and a spectrum of different water relaxators[10] this behavior must produce an inflection point in the ϵ_2 response of the samples, which, however, is not found. Besides that, such steplike increases of at least a factor of 3 for the dipole relaxation frequency of water molecules were never observed in lipid/water mixtures at the chain melting phase transition. On the contrary, in all our mixtures with water concentrations from 2 to 67 water molecules per lipid molecule, a slight decrease (about 1%) was measured.[10]

So we conclude that the chain melting produces an additional dielectric dispersion at microwave frequencies. The dispersion is based on the electric dipole moment of an excited *gauche* conformation in an acyl chain. Its value depends on the conformation type and has a value between 0.03 to 0.06 debye, which was found by quantum-mechanical calculations.[12] The dipole moment of propane was measured to be 0.083 debye.[13] This value represents an upper limit for asymmetric conformations in saturated acyl chains.[12]

Table I gives the positive contributions of the chain melting signal to the dielectric constant at the phase transition and some corresponding thermodynamic data. For various mixtures both $\Delta\epsilon_1$ and $\Delta\epsilon_2$ of the chain melting steps are listed; for some mixtures only the $\Delta\epsilon_2$ values were resolvable. The former are accessible to a Debye relaxation analysis, for which a single relaxation process was assumed.[11] The evaluated contributions of the chain melting to the static dielectric constant $(\Delta\epsilon_s)_s$ of the samples are listed, and in the next column values are given for the changes in $(\Delta\epsilon_s)_c$ for only the excited chains. The latter are given considering the volume fractions of the excited chain parts in the sample and the incorporation of these parts into the head group regions, which have the same dielectric constant as the unexcited chains. This is exact for sample (1), whereas with increasing water content the values of $(\Delta\epsilon_s)_c$ may be a little too high due to internal depolarizing fields. These arise from water regions in the samples having a higher dielectric constant than the bilayer regions.

A calculation was performed by the Kirkwood formula (1) to evaluate the dielectric constant $(\Delta\epsilon_s)_c$ of the excited chains assuming a dipole moment for a *gauche* conformation of $\mu = 0.08$ D (the dipole density N_0 was evaluated from the volume fraction and an average excitation of three *gauche* conformations per chain, the polarizability of the chains is $\epsilon_\infty = 2$, T is the temperature in K, k_B is Boltzmann's constant). The Kirkwood correlation factor $(1 + z \overline{\cos\gamma})$ describes the interaction between the molecular dipoles (z is the number of neighboring dipoles, $\overline{\cos\gamma}$ is the mean cosine of the angle between their vectors).

$$\epsilon_s - \epsilon_\infty = \frac{3\epsilon_s}{2\epsilon_s + \epsilon_\infty} \frac{4\pi N_0}{3k_B T} \mu^2 (1 + z \overline{\cos\gamma}) \ . \tag{1}$$

It follows from this relation that the Kirkwood correlation factor must at least exceed values of 250 to reproduce the measured values of $(\Delta\epsilon_s)_c$. This means that the trans-gauche isomerization rotation in the fluid phase is highly correlated over the same quantity of *gauche* conformations with parallel alignment, i.e., a cooperative rotational flipping motion over rather large clusters of neighboring CH_2-CH_2 bonds. In the case of nonparallel simultaneous motion the clusters are even larger, just as if the dipole moment of a *gauche* conformation has a smaller value than the supposed 0.08 D, which is an upper limit.[12]

Recently, such a behavior has been required by a dynamic theory of chain melting in phospholipid membranes by Holz, Vigren, and Zuckermann (HVZ).[7] There the constraint of chain pairing due to the lateral pressure in a membrane was incorporated in the process of dislocation-mediated melting in a quasi-two-dimensional system. The basis for such a theory is the experimental observation that the density of phospholipid bilayers decreases by only 4% at the chain melting phase transition,[14] whereas the bilayer thickness decreases for about 10%.[15] Consequently, the chains do not gain much additional space, so a cooperative rotational motion is compelling.

Hints of such a behavior were given in the past by NMR–relaxation-time and order-parameter measurements, both of which yield a plateau from the C_2 to the C_8 segments of the acyl chains in DPPC [the lecithin with saturated C_{16} chains; 1,2-dipalmitoyl-(S-glycero-3-phosphocholine)], whereas the relaxation times and order parameters of the segments $C_{10}-C_{16}$ decrease.[16,17] This implies a constant interaction for C_2 to C_9, so that the motion in this region is assumed to be more cooperative than that of the end segments.

Our dielectric data present the first experimental evidence for a large-scale motion correlation, and at the same time they provide a lower limit for the corresponding cluster size of about 250 simultaneously rotating CH_2-CH_2 bonds. According to the NMR data on DPPC (Refs. 16 and 17) and the HVZ theory[7] the lattice interaction is constant for the acyl segments C_2 to at least C_{10} of DMPC in the fluid phase. Considering this fact, we conclude that the correlated rotation between neighboring chains, and consequently the ob-

served dielectric response of the chain melting occur in this region. From the Debye analysis, the dielectric relaxation frequencies f_D were estimated. The corresponding relaxation times τ_D of $1.6-8\times10^{-11}$ s are in good agreement with the NMR correlation times τ_c of DPPC,[16] considering the relation $\tau_c=\frac{1}{3}\tau_D$.[18] At different measurement frequencies, however, variations in relaxation frequency and static dielectric constant were also observed in the same sample. Therefore, we assume that there are different types of correlated rotation with distinct relaxation times τ_D and cluster sizes. In this case a Debye analysis must consider a distribution or even more complicated dielectric relaxation processes according to recent theories.[19] Yet we do not have sufficient data to analyze the relaxation spectrum. Such a spectrum of motion relaxation times for one CH_2-acyl-chain segment is not amenable to the averaging NMR–correlation-time measurements.

Table I shows the influence of hydration on thermodynamic parameters of the chain melting, such as transition temperature and transition width. The hydration rate influences the lateral pressure in the bilayer and the packing geometry, and so alters the lattice conditions inside the acyl chains.[20,21]

It is difficult to get information about the temperature dependence of the correlation effects in the fluid phase, because the development of the dielectric response is mainly determined by the properties of the water molecules in the samples. However, above the chain melting transition the evaluation of the temperature-dependent static dielectric constant ϵ_s of the samples shows a slight increase in all samples with increasing temperature (again evaluated by a single Debye relaxation analysis). Consequently, the correlation effects cannot decrease very much in the fluid phase with increasing temperature.

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