Bending elasticity of saturated and monounsaturated phospholipid membranes studied by the neutron spin echo technique

2009 J. Phys.: Condens. Matter 21 155104

(http://iopscience.iop.org/0953-8984/21/15/155104)

IOP Publishing Journal of Physics: Condensed Matter

J. Phys.: Condens. Matter **21** (2009) 155104 (7pp) doi:10.1088/0953-8984/21/15/155104

Bending elasticity of saturated and monounsaturated phospholipid membranes studied by the neutron spin echo technique

Zheng Yi[1,2], Michihiro Nagao[2,3] and Dobrin P Bossev[1]

[1] Physics Department, Indiana University, Bloomington, IN 47405, USA
[2] Center for Neutron Research, National Institute of Standards and Technology, Gaithersburg, MD 20899, USA
[3] Cyclotron Facility, Indiana University, Bloomington, IN 47408, USA

E-mail: dbossev@indiana.edu

Received 9 December 2008, in final form 4 February 2009
Published 20 March 2009
Online at stacks.iop.org/JPhysCM/21/155104

Abstract

We have used neutron spin echo (NSE) spectroscopy to study the effect of bilayer thickness and monounsaturation (existence of a single double bond on one of the aliphatic chains) on the physical properties of unilamellar vesicles. The bending elasticity of saturated and monounsaturated phospholipid bilayers made of phospholipids with alkyl chain length ranging from 14 to 20 carbons was investigated. The bending elasticity κ_c of phosphatidylcholines (PCs) in the liquid crystalline (L_α) phase ranges from 0.38×10^{-19} J for 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine to 0.64×10^{-19} J for 1,2-dieicosenoyl-*sn*-glycero-3-phosphocholine. It was confirmed that, contrary to the strong effect on the main transition temperature, the monounsaturation has a limited influence on the bending elasticity of lipid bilayers. In addition, when the area modulus K_A varies little with chain unsaturation or length, the elastic ratios $(\kappa_c/K_A)^{1/2}$ of saturated and monounsaturated phospholipid bilayers varies linearly with lipid hydrophobic thickness d which agrees well with the theory of ideal fluid membranes.

1. Introduction

Biomembranes of natural living cells are made of bilayers of phospholipid molecules embedded with other constituents, such as cholesterol and membrane proteins, which help to accomplish a broad range of functions [1]. Since the lipid bilayers are characterized by a distinct central hydrophobic region sandwiched by two polar interfaces, we strive to understand how the configuration of aliphatic chains in the hydrophobic region influences the biological, physical and chemical properties of biomembranes. In this paper we report bending elasticities of saturated and monounsaturated phospholipids studied by the neutron spin echo (NSE) technique.

The bending elasticity is an important mechanical property that governs the thermal fluctuations of bilayers, gives rise to undulation forces and predetermines the contact time of the membranes with solid substrates and other objects [2]. Here we have attempted to elucidate the influence of monounsaturation and the thickness of the hydrophobic region d on the bending elasticity κ_c of artificial phospholipid membranes that form large vesicles. Membranes made of saturated phospholipids undergo a first-order melting transition, the so-called main transition from the ripple gel (P'_β) phase to the liquid crystalline (L_α) phase at temperatures above the freezing point of water [3]. Although the lower transition temperatures can be achieved by having shorter chain length in saturated lipids, the introduction of the *cis* double bond drastically decreases the main transition temperatures without changing the chain length, thus ensuring the fluidity and temperature insensitivity of biomembranes [4–6]. The double bond exists in the normal *cis* configuration which is

asymmetric and forces a kink of the hydrocarbon chain. As a result the unsaturated lipids are packed together loosely, or crystallize less readily compared to the straight chain saturated lipids. In agreement with this scenario, recent studies by x-ray scattering have found that the areas per lipid molecule of monounsaturated lipids are larger than those of the corresponding saturated lipids [6] with the same chain length. The effect of the double bond on transition temperatures has been referred to when the bilayers with double bonds are often described as being more 'fluid' than the corresponding fully saturated bilayers at the same temperature [5]. In the scope of our paper, the membrane 'fluidity', which is frequently related to the packing of the hydrocarbon chains, is different from the definition of the membrane bending elasticity κ_c. We have addressed the question of whether the introduction of a single double bond can affect the bending elasticity in a similar manner to the membrane fluidity.

The main transition temperature of saturated phospholipid bilayers is also known to be proportional to the length of the alkyl chains, from 24 °C for 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (14:0 PC) with 14 carbon atoms and zero double bonds, to 64 °C for 1,2-diarachidoyl-*sn*-glycero-3-phosphocholine (20:0 PC) with 20 carbon atoms and zero double bonds [7, 8]. Similarly, monounsaturated lipid bilayers also show such dependence [9]. This dependence originates from the balance between the interaction energy of the headgroups, which is constant, and the total interaction between the chains, which decreases with the alkyl chain length [10]. Furthermore the change of hydrophobic thickness also has been linked to such properties as ion permeability [11, 12], regulation of a membrane protein function [13, 14] and electric capacitance [15]. However, how to measure directly the effect of the hydrophobic thickness on thermal undulations of biomembranes without using external forces remains an open question.

Most of the work on determination of the bending elasticity of phospholipid bilayers is based on static structural methods such as small angle x-ray and neutron scattering techniques [18, 19]. These methods determine the time averaged roughness of the lipid layers that can be related to κ_c through a model. Nuclear magnetic resonance (NMR) spin–spin relaxation time can also be used to determine κ_c by probing the dynamics of the bilayer; however, this method involves speculative assumptions and is quite indirect [20, 21]. Here we have chosen neutron-spin-echo (NSE) spectroscopy as the most suitable method to determine κ_c. NSE is a dynamic method, ideal for studies of the thermal fluctuations of the biomembranes because its correlation times (0.1 ns to 100 ns) and length scales (10 Å to 10^3 Å) overlap with those of the cell membrane fluctuations [22, 23]. In this paper the bending elasticity of several saturated and monounsaturated phospholipids with the same hydrocarbon numbers were measured by the NSE technique. The intermediate scattering function acquired through NSE was analyzed by the Zilman–Granek model [24, 25] for two-dimensional (2D) free-standing membranes. We have explored the question of how the presence of the double bond and hydrophobic thickness affect the bending elasticity of lipid bilayers in the L_α phase.

2. Materials and methods

2.1. Lipids and vesicle preparation

Seven synthetic species of diacyl-PC lipids from Avanti Polar Lipids (Alabaster, AL) were used without further purification. The following three were fully saturated 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (14:0 PC); 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine(16:0 PC) and 1,2-distearoyl-*sn*-glycero-3-phosphocholine (18:0 PC), with chain length from 14 to 18 carbon atoms. The following four were *cis* unsaturated 1,2-dimyristoleoyl-*sn*-glycero-3-phosphocholine (14:1 PC); 1,2-dipalmitoleoyl-*sn*-glycero-3-phosphocholine (16:1 PC); 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (18:1 PC) and 1,2-dieicosenoyl-*sn*-glycero-3-phosphocholine (20:1 PC). The vesicles were prepared by the extrusion method [26]. To ensure good mixing and dispersion, the weighed amount of lipids was dissolved in HPLC grade chloroform (Fluka). Then the chloroform was evaporated by a stream of dry N_2 gas. Any solvent residuals were further removed by placing the sample in a vacuum chamber at 60 °C for 30 min. The necessary amount of deuterated water D_2O (either from CDN isotopes or Cambridge isotopes) was added to achieve final concentration of a mass fraction of 2.0%. Deuterated water was used as a solvent to keep the incoherent background low and to provide high scattering contrast. The mixtures were vortexed on a Daigger Vortex for about 10 min until all the lipids were suspended and the resulting lipid suspensions were repeatedly filtered through a Nalgene filter with a pore size of 400 nm that predetermined the average diameter of the vesicles. The vesicle size distribution was characterized on a dynamic light scattering (DLS) DynaPro-Titan system equipped with an He–Ne ion laser ($\lambda = 783$ nm). The DLS method yields the normalized intensity time correlation function that was converted to relaxation time by an inverse Laplace transformation. The diffusion coefficient was obtained from the moments of the time distribution of the intensity and the hydrodynamic radius of the vesicles was finally obtained using the Stokes–Einstein equation [27]:

$$R_H = \frac{k_B T}{6\pi \eta_0 D} \tag{1}$$

where D is the mean diffusion coefficient, k_B is the Boltzmann constant, T is the absolute temperature and η_0 is the solvent viscosity. The averaged radii of the vesicles were about 200 nm, and the radius distribution function was shown in a previous paper [26].

2.2. NSE spectroscopy

The NSE technique has been widely used in the study of dynamic processes in macromolecular systems that are relevant to, among others, polymer and biomedical sciences [28–30]. Our data were taken on the spectrometer located on the NG5 guide of the NIST Center for Neutron Research (NCNR) [31]. The momentum transfer Q (scattering vector) ranged from 0.03 to 0.12 $Å^{-1}$. The longer wavelength of 11.6 Å was chosen to access the lower regions of the scattering vector at

the expense of reduced beam intensity. The reduced NSE data were analyzed using DAVE software provided by NCNR [32] as reported elsewhere [26]. The temperature was controlled by an oil circulation bath with the accuracy of ±0.1 °C. We measured the temperature dependence of the bending elasticity of several saturated lipids 14:0 PC, 16:0 PC and 18:0 PC for the purpose of testing the temperature component of the model and to obtain comparative values for the monounsaturated lipids. The monounsaturated lipid membranes 14:1 PC, 16:1 PC, 18:1 PC and 20:1 PC were measured at temperatures above the main transitions.

The shape fluctuations of the vesicles dominate at the Q range corresponding to the dip position in the small angle scattering; in the present case, we saw a dip at around $Q \approx 0.003$ Å^{-1}, which is below the accessible Q range of the NSE instrument. The accessible Q range of the NSE spectrometer corresponds to length scales that are an order of magnitude smaller than the radius of the vesicles (about 200 nm) hence the vesicle deformations do not have an effect on the intensity decay. The intermediate scattering function $I(Q,t)$ can be solely attributed to the surface undulations with wavevectors much smaller than the vesicle radius. Thus we have chosen the Zilman–Granek model for free-standing membranes to explain the NSE decay.

2.3. Zilman–Granek model

The membrane dynamics in the lamellar phase could be qualitatively explained by the theory given by Zilman and Granek [24, 25]. The time decay of $I(Q,t)$ originating from thermal undulations of a free-standing (unsupported) single membrane can be predicted to exhibit stretched exponential decay:

$$I(Q,t) = I(Q,0)\mathrm{e}^{-(\Gamma t)^{\alpha}}, \tag{2}$$

where Γ is the relaxation rate, and α is the exponential stretching factor which is given by

$$\alpha = \frac{2}{3}\left(1 + \frac{k_{\mathrm{B}}T}{4\pi\kappa_c}\right), \tag{3}$$

where κ_c is the bending elasticity. For lipid bilayers, $\kappa/k_{\mathrm{B}}T$ is expected to be of the order of 10 or greater, which is supported by experimental findings and predicted by computer simulation. At $\kappa/k_{\mathrm{B}}T \gg 1$ the factor α is close to 2/3 and it is the case of the present system, hence we have fitted $I(Q,t)$ according to equation (2) with $\alpha = 2/3$ to determine Γ as a function of Q.

The relaxation rate Γ is related to the bending elasticity as

$$\Gamma = 0.025\gamma_k \left(\frac{k_{\mathrm{B}}T}{\kappa_c}\right)^{\frac{1}{2}} \frac{k_{\mathrm{B}}T}{\eta} q^3, \tag{4}$$

where η is the viscosity of the solvent water and the factor γ_k originates from averaging over the angle between the wavevector and the plaquette surface normal. The parameter γ_k approaches unity when $\kappa/k_{\mathrm{B}}T \gg 1$. In this analysis, we assumed the solvent viscosity as three times the value of averaged viscosity of D_2O ($\eta = 3\eta_{\mathrm{D2O}}$) for viscosity η when taking the local dissipation at the membrane into consideration [33, 34]. The viscosity of D_2O was taken as 0.973×10^{-3} Pa s and 0.553×10^{-3} Pa s at 30 °C and 60 °C, respectively. Figure 1 shows $I(Q,t)/I(Q,0)$ obtained by NSE against the Fourier time t at different Q values ranging from 0.05 Å^{-1} to 0.12 Å^{-1} for 14:0 PC, 16:0 PC, 18:1 PC and 20:1 PC vesicles in the L_α phase. The solid lines represent fits to equation (2). The nice fitting confirmed that the NSE decay reflects predominantly the thermal fluctuations exhibited by the lipid bilayers. The Zilman–Granek's model fitted the scattering data very well, hence the slight differences between the conditions for saturated and unsaturated lipids can be neglected within the precision of the experiment.

It has been shown by Mihailescu *et al* [35] that Zilman–Granek model involves inaccurate approximations to yield precise absolute values for the bending elasticity; however, one can obtain relative values from equation (4) that can be very informative. In order to obtain reasonable values of bending elasticity, it was necessary to assume the viscosity η to be three times the value of the viscosity of D_2O. Such an assumption was applied by Farago *et al* [33] and Takeda *et al* [34] to explain the results for the bicontinuous microemulsion and the lamellar phases of the $C_{12}E_5$/n-octane/water system and also for the lamellar phase of the DPPC/water/$CaCl_2$ system. They thought that the fitted viscosity was due to the local dissipation around amphiphile membranes. Recently, Komura *et al* [36, 37] analyzed their NSE data on ternary microemulsion systems in terms of three models, including Zilman–Granek, and they showed that the viscosity could be a different value depending on the model. They suggested that the effective η should be from three to four times the mean values of the solvent viscosity in the Zilman–Granek model.

3. Results and discussion

The calculated relaxation rate Γ as a function of Q in a double logarithmic plot of 16:0 PC at 30 °C, 41 °C and 60 °C is shown in figure 2 where the solid lines are fits to equation (4). When fitted by a power law with a variable exponential factor we have found the slope to vary within 2.6 to 3.4 which is close to 3 as predicted by equation (4). This confirms the Q^{-3} dependence of the relaxation rate and indicates that the NSE result is directly related to the membrane undulation since it follows Zilman and Granek's prediction.

We have calculated the bending elasticity from the scattering intensity using equation (4). It is known that at $T \gg T_{\mathrm{m}}$ the temperature has a minimal effect on the properties of the lipid bilayers in the L_α phase. The bending elasticity κ_c also is expected to be independent of temperature. To examine the effect of temperature on κ_c of saturated lipids we examined vesicles made of 14:0 PC, 16:0 PC and 18:0 PC across their melting temperatures (table 1).

To analyze the temperature dependence we have plotted κ_c against $T - T_{\mathrm{m}}$ in figure 3. It is seen that when the phospholipids are in the L_α phase, κ_c is independent of the temperature. It is noted that the value of κ_c is slightly larger in the vicinity of the main transition temperature. This temperature corresponds to the anomalous swelling regime and

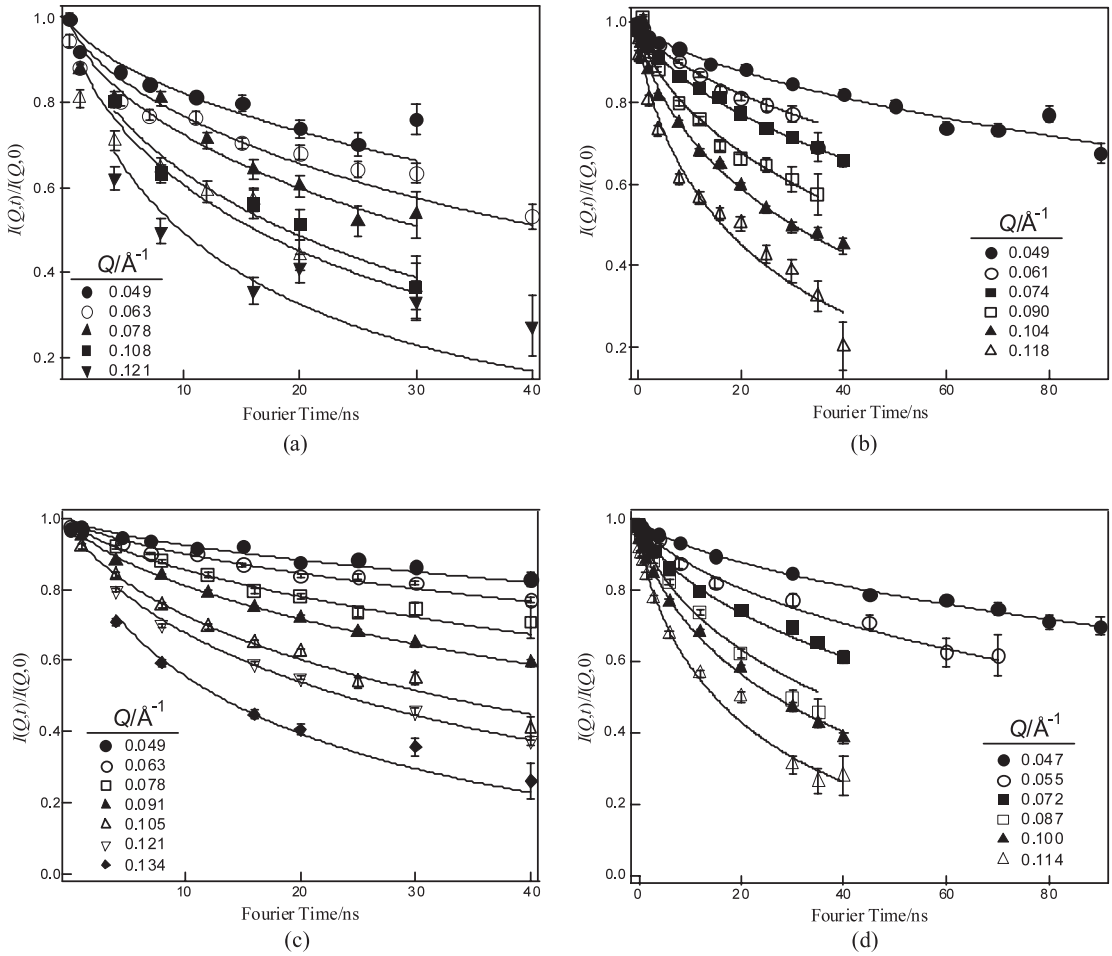

Figure 1. Normalized intermediate scattering functions against Fourier time obtained by NSE at different Q values for vesicles made of different phospholipids in D_2O at 60 °C: (a) 18:1 PC; (b) 20:1 PC; (c) 14:0 PC and (d) 16:0 PC. The error bars shown indicate ± one standard deviation. Note the longer Fourier time scales in (b) and (d).

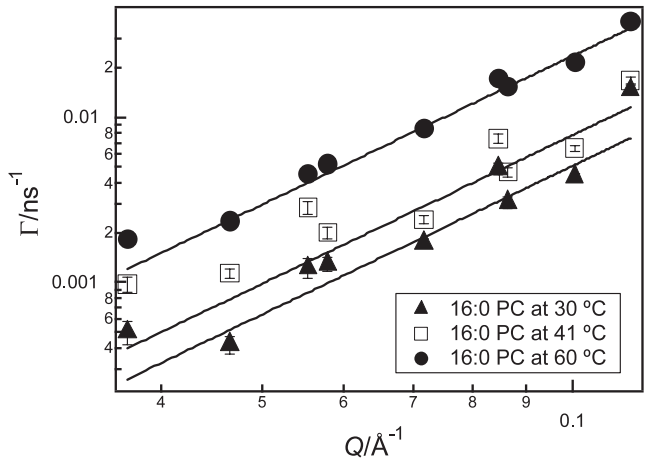

Figure 2. Relaxation rate Γ for 16:0 PC in D_2O at 30, 41 and 60 °C as a function of the scattering vector Q. The solid lines are fits to equation (4).

Table 1. Bending elasticity κ_c of saturated bilayers in D_2O calculated from equation (4) at different temperatures.

Lipid	T_m (°C)	T (°C)	$(T - T_m)$ (°C)	κ_c/k_BT
14:0 PC	24	22	−2	100.0 ± 4.99
		24	0	20.9 ± 0.61
		28	+4	13.9 ± 0.24
		35	+11	15.3 ± 0.31
		45	+21	13.9 ± 0.44
		60	+36	8.2 ± 0.12
16:0 PC	41	30	−11	49.6 ± 2.78
		41	0	36.1 ± 1.49
		60	+19	9.5 ± 0.18
18:0 PC	54	40	−14	79.1 ± 3.23
		60	+6	13.6 ± 0.24

the present NSE result is consistent with the result obtained by Seto *et al* [38]. At temperatures below the main transition temperature κ_c dramatically increases. The measured κ_c in the P'_β phase of all three kinds of saturated lipid bilayers are 6 to 10 times κ_c in the L_α phase. The high values of κ_c in the P'_β phase mean significant stiffening of the lipid bilayers which can be explained by the higher ordering of the lipid molecules in the P'_β phase than in the L_α phase.

To understand the specific effect of the double bond on bending elasticity of lipid bilayers we measured the bending elasticities of several saturated and monounsaturated lipid bilayers with the same hydrocarbon numbers in the L_α state.

Table 2. Bending elasticity κ_c of saturated and monounsaturated bilayers in D_2O calculated from equation (4) at different temperatures. K_A, d and A are quoted from [10], [16] and [6], respectively.

Lipid	κ_c (10^{-19} J)	T (°C)	κ_A (N m^{-1})	d (nm)	A (Å^2)	β
14:0 PC	0.38 ± 0.01	60	0.234	2.30 ± 0.1	60.2	$1/(26.9 \pm 0.76)$
16:0 PC	0.44 ± 0.01	60	0.234	2.60 ± 0.1	59	$1/(35.9 \pm 0.87)$
18:0 PC	0.62 ± 0.01	60	0.235	2.95 ± 0.1	57	$1/(32.9 \pm 0.57)$
14:1 PC	0.45 ± 0.02	30	0.250	2.00 ± 0.1	76	$1/(22.2 \pm 1.04)$
16:1 PC	0.38 ± 0.02	30	0.250	2.35 ± 0.1	74	$1/(36.3 \pm 1.98)$
18:1 PC	0.41 ± 0.02	60	0.236	2.70 ± 0.1	72	$1/(41.7 \pm 2.09)$
20:1 PC	0.73 ± 0.02	60	0.250	3.05 ± 0.1	70	$1/(31.8 \pm 0.91)$

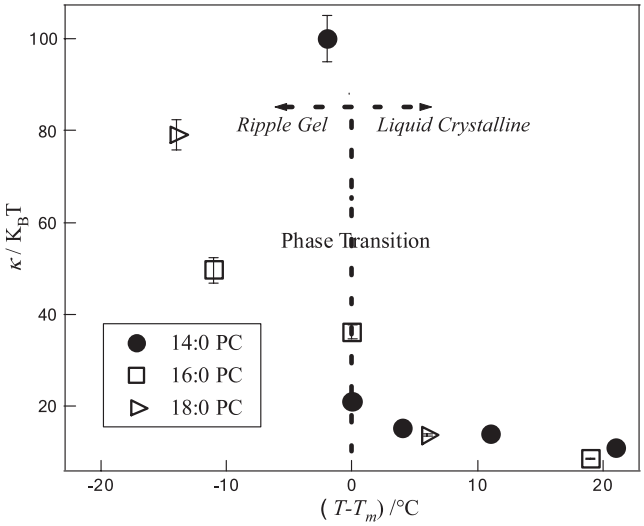

Figure 3. The bending elasticity κ_c of 14:0 PC, 16:0 PC and 18:0 PC bilayers in D_2O plotted against $(T - T_m)$.

All samples were measured at 60 °C except for 14:1 PC and 16:1 PC where the data were taken at 30 °C because of their extremely low main transition temperatures. The calculated relaxation rate Γ as a function of Q of monounsaturated and saturated phospholipid bilayers is shown in table 2 and plotted in a double logarithmic plot in figure 4.

We found that κ_c values for 14:1 PC, 16:1 PC and 18:1 PC are very similar to those of 16:0 PC and 18:0 PC. This means that the structural changes caused by the double bond, reducing the hydrophobic thickness and increasing the area per molecule all lead to decreasing of the bending elasticity. This is contrary to the effect on the membrane fluidity which is strongly dependent on the temperature and shows phase transition-like behavior. The influence of the double bond on bending elasticity is very limited. As the main transition temperature is greatly related to the ordering of the hydrophobic parts, the kink in the hydrocarbon chain caused by a double bond changes the packing order of the phospholipid bilayers. Since the bending elasticity is related to the undulation of the membrane tiny structural changes of phospholipid conformations only give rise to small changes in the dynamics of biomembranes. Our results show that the bending elasticity of the monounsaturated bilayer in the liquid crystalline phase is slightly smaller than that of the saturated one. However, the monounsaturation has a strong effect on the transition temperature, which suggests that it is possible to reduce the transition temperatures of the living membranes by the presence of monounsaturated or even polyunsaturated lipid bilayers without changing the total lipid bilayers' mechanical properties.

We can find from table 2 that the bending elasticities of either monounsaturated or saturated lipid bilayers fluctuate irregularly with the increase of the hydrocarbon number within the experimental error. The values of κ_c seem to be independent of the number of carbon atoms in the hydrophobic chain. From the mechanics of a thin slab of solid material it is known

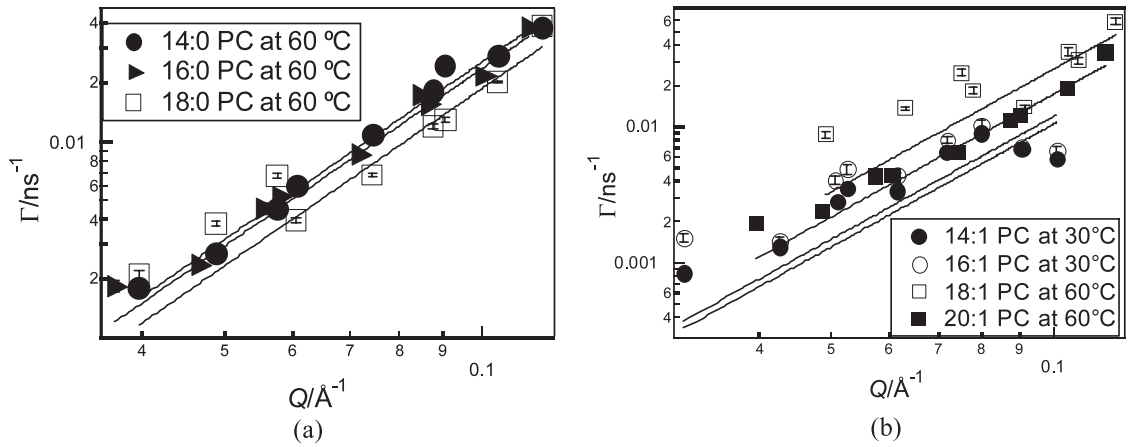

Figure 4. Relaxation rate Γ of membranes in the L_α phase made of saturated (a) and monounsaturated phospholipids (b) as a function of the scattering vector Q. The respective lipids are identified in the legend and the solid lines are fits to equation (4).

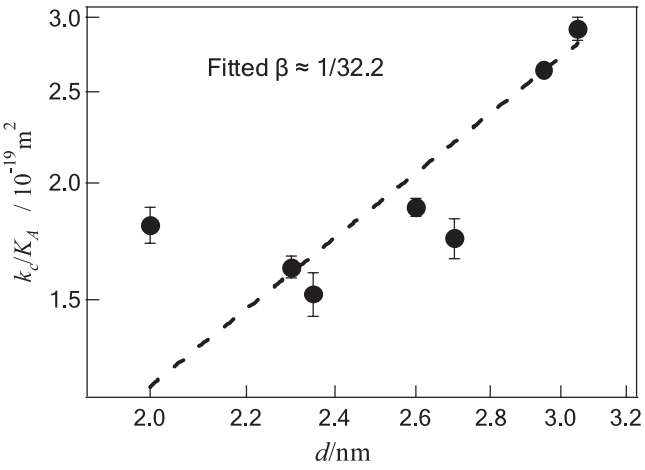

Figure 5. The ratio κ_c/K_A for 14:0 PC, 16:0 PC, 18:0 PC, 14:1 PC, 16:1 PC, 18:1 PC and 20:1 PC plotted against hydrophobic thickness d. The dashed line is a least-square fit to $\kappa_c = \beta K_A d^2$. The slope of this curve is approximately equal to $(1/32.2)^{1/2}$ (see the text).

that the bending elasticity should scale as the area modulus K_A multiplied by the square of the thickness d^2:

$$\kappa_c = \beta K_A d^2 \tag{5}$$

where β is a normalization constant. For fluid membranes it has been shown that K_A varies little with chain unsaturation or its length [5]. We speculate that the variations in bending elasticity κ_c in different phospholipid bilayers are due to the differences in bilayer hydrophobic thickness. As a test of this hypothesis we have plotted in figure 5 the elastic ratio κ_c/K_A as a function of the hydrophobic thickness d in a double logarithmic plot. The slope of this curve fitting $(\beta_A)^{1/2}$ is approximately equal to $(1/32.2)^{1/2}$, which agrees well with the results of Rawicz *et al* [16] and Bermudez *et al* [17] obtained by micropipette pressurization [39, 40]. The area moduli K_A and hydrophobic thicknesses d for these seven lipid bilayers were quoted from [16] (K_A of 14:1, 16:1 and 20:1 PCs were assumed as the averaged K_A of phospholipid bilayers) and are also listed in table 2 for comparison. The normalization constants β were calculated from equation (5). Compared to the β_A obtained from the fits to equation (5), all the β of seven lipid bilayers vary from 1% (for 20:1 PC) to 23% (for 18:1 PC). The ideal fitting in figure 5 shows that in a broad range of chain lengths (from 14 to 20), either with or without the presence of one double bond on the hydrophobic chain, the bending elasticity of lipid bilayers is quadratically dependent on the hydrophobic thickness. For fluid membranes κ_c is controlled by two independent parameters K_A and d. On the other hand K_A is independent of the hydrophobic thickness d and depends strongly on the interfacial structures. The prefactor β can be assumed to be a constant that is not influenced by K_A and d. However, it was noted in others papers [7] that for polymer vesicles β becomes relatively large because of the high viscosity and possibly entangled membranes.

4. Conclusions

We have used neutron spin echo spectroscopy (NSE) to measure the dynamics of unilamellar vesicles made from saturated and monounsaturated phospholipid bilayers with different numbers of hydrocarbons in hydrophobic chains in both P'_β and L_α phases. Unlike the P'_β-to-L_α transition temperature, the bending elasticity κ_c in L_α phases is weakly affected by the presence of one double bond in one of the hydrophobic chains. This softness caused by the monounsaturation originates mainly from the change of the hydrophobic thickness. We also examined the relationship of the bending elasticity against the square of the hydrophobic thickness: $\kappa_c = \beta K_A d^2$. Since there is a small change in area moduli K_A in fluid membranes, the strong variations in bending elasticities are only due to differences in bilayer hydrophobic thickness. The prefactor β can be assumed to be a constant which is not influenced by the stretching modulus and the bilayer hydrophobic thickness.

Acknowledgments

This work utilized facilities supported in part by the National Science Foundation under Agreement No. DMR-0454672. We acknowledge the support of the National Institute of Standards and Technology, US Department of Commerce, in providing the neutron research facilities used in this work. We thank Antonio Faraone for valuable discussions and his help with the data collection at NCNR and Larry Kneller for his support in our data analysis.

Certain trade names and company products are identified in order to specify adequately the experimental procedure. In no case does such identification imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the products are necessarily the best for the purpose.

References

[1] Stoecken W and Engelman D M 1969 *J. Cell Biol.* **42** 613
[2] Duwe H P and Sackmann E 1990 *Physica* A **163** 410
[3] Cevc G 1987 *Biochemistry* **26** 6305
[4] Cevc G 1991 *Biochemistry* **30** 7186
[5] Seelig A and Seelig J 1977 *Biochemistry* **16** 45
[6] Kucerka N *et al* 2005 *J. Membr. Biol.* **208** 193
[7] Ladbrooke B D and Chapman D 1969 *Chem. Phys. Lipids* **3** 304
[8] Chapman D 1965 *The Structure of Lipids* (London: Methuen)
[9] Caffrey M and Feigenson G W 1981 *Biochemistry* **20** 1949
[10] Lewis B A and Engelman D M 1983 *J. Mol. Biol.* **166** 211
[11] Aguilella V, Belaya M and Levadny V 1996 *Langmuir* **12** 4825
[12] Matsuoka S and Murata M 2003 *Biochim. Biophys. Acta* **1617** 109
[13] Lewis B A and Engelman D M 1983 *J. Mol. Biol.* **166** 203
[14] Anderson O S and Roger R E II 2007 *Annu. Rev. Biophys. Biomol. Struct.* **36** 107
[15] Ashcroft R G *et al* 1981 *Biochim. Biophys. Acta* **643** 191
[16] Rawicz W *et al* 2000 *Biophys. J.* **79** 328
[17] Bermudez H *et al* 2004 *Langmuir* **20** 540
[18] Daillant J *et al* 2005 *Proc. Natl Acad. Sci. USA* **102** 11639

[19] Lemmich J *et al* 1996 *Phys. Rev.* E **53** 5169
[20] Dave P *et al* 2004 *Biophys. J.* **86** 1564
[21] Eisenblätter J and Winter R 2006 *Biophys. J.* **90** 956
[22] Takeda T *et al* 1999 *J. Phys. Chem. Solids* **60** 1375
[23] Yamada N *et al* 2005 *J. Phys. Soc. Japan* **74** 2853
[24] Zilman A and Granek R 1996 *Phys. Rev. Lett.* **77** 4788
[25] Zilman A and Granek R 2002 *Chem. Phys.* **284** 195
[26] Yi Z and Bossev D P 2007 *QENS2006 Conf. Proc.* p 123
[27] Brown W 1993 *Dynamic Light Scattering. The Method and Some Applications* (Oxford: Clarendon)
[28] Yardmici H *et al* 2005 *J. Chem. Phys.* **123** 244908
[29] Rheinstadter M C *et al* 2006 *Phys. Rev. Lett.* **97** 048103
[30] Monkenbusch M *et al* 2005 *J. Phys.: Condens. Matter* **17** 2903
[31] Rosov N, Rathgeber S and Monkenbusch M 2000 *Scattering from Polymers: Characterization by X-Rays, Neutrons and Light* (*ACS Symp. Series* vol 739) ed P Cebe, B S Hsiao and D J Lohse (Washington, DC: American Chemical Society) pp 103–16
[32] http://www.ncnr.nist.gov/dave
[33] Farrago B *et al* 1995 *Physica* B **213** 712
[34] Takeda T *et al* 1999 *J. Phys. Chem. Solids* **60** 1375
[35] Mihailescu M *et al* 2002 *Phys. Rev.* E **66** 041504
[36] Komura S, Takeda T, Kawabata Y, Ghosh S K, Seto H and Nagao M 2001 *Eur. Phys. J.* E **5** 329
[37] Komura S, Takeda T, Kawabata Y, Ghosh S K, Seto H and Nagao M 2001 *Phys. Rev.* E **63** 041402
[38] Seto H, Yamada N L, Nagao M, Hishida M and Takeda T 2008 *Eur. Phys. J.* E **26** 217
[39] Kwok R and Evans E 1981 *Biophys. J.* **35** 637
[40] Evans E and Needham D 1987 *J. Phys. Chem.* **91** 4219