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Phosphatidylcholine 'Wobble' in Vesicles Assessed by High-Resolution ^{13}C Field Cycling NMR Spectroscopy

V.N. Sivanandam[§,†], **Jingfei Cai**[‡], **Alfred G. Redfield**[†], and **Mary F. Roberts**[‡,*]

[†]Department of Chemistry and Biochemistry, Brandeis University, Waltham, Massachusetts 02454

[§]Rosenstiel Basic Medical Sciences Research Institute, Brandeis University, Waltham, Massachusetts 02454

[‡]Department of Chemistry, Boston College, Chestnut Hill, Massachusetts 02467

Abstract

High resolution ^{13}C NMR field cycling (covering 11.7 down to 0.002 T) relaxation studies of the sn-2 carbonyl of phosphatidylcholines in vesicles provide a detailed look at the dynamics of this position of the phospholipid in vesicles. The spin-lattice relaxation rate, R_1, observed down to 0.05 T is the result of dipolar and CSA relaxation components characterized by a single correlation time τ_c, with a small contribution from a faster motion contributing CSA relaxation. At lower fields, R_1 increases further with a correlation time consistent with vesicle tumbling. The τ_c is particularly interesting since it is 2-3 times slower than what is observed for ^{31}P of the same phospholipid. However, cholesterol increases the τ_c for both ^{31}P and ^{13}C sites to the same value, ~25 ns. These observations suggest faster local motion dominates the dipolar relaxation of the ^{31}P while a slower rotation or wobble dominates the relaxation of the carbonyl carbon by the α-CH_2 group. The faster motion must be damped with the sterol present. As a general methodology, high resolution ^{13}C field cycling may be useful for quantifying dynamics in other complex systems as long as a ^{13}C label (without attached protons) can be introduced.

Motions of phospholipids in membranes have been the subject of intensive research for several decades. While chain motions are understood in a quantitative fashion as a result of diverse ^{2}H and ^{13}C NMR studies,[1] the dynamic behavior of the interfacial region, including the fatty acyl carbonyls and the phosphorus moiety, is much less well characterized. Spin-lattice relaxation rates (R_1) measured over a range of magnetic fields can in principle be very useful for determining time scales for different motions in the lipid bilayer. However, for phospholipid aggregates only a few studies have measured R_1 (^{2}H and ^{13}C) at three or four fields, and those above 1 T, to try and assess lipid motions.[2,3] Recent high-resolution ^{31}P field cycling spin-lattice relaxation studies have yielded information on the motions of the phosphorus of different phospholipids in diverse aggregates.[4-6] In this technique, the magnetic field is cycled by mechanically shuttling the sample from the center of the probe to a substantially lower magnetic field above the probe for the delay times normally used in conventional NMR relaxation sequences, then shuttling the sample back to the probe for readout of the relaxation at the lower field. This method, which allows access to a very wide field range (0.002 to 11.7T) for measuring R_1 and interpreting it in terms of spectral density functions,[4,5] is particularly useful for spin ½ nuclei without a directly bonded proton where the relaxation at the high fields of modern spectrometers is moderately long (R_1 ~1 s^{-1}) and dominated by mechanisms other than dipole-dipole interactions (e.g., chemical shift anisotropy, CSA). For multicomponent phospholipid vesicles, the analysis of the ^{31}P R_1 profile

mary.roberts@bc.edu.

as a function of field for each phospholipid molecule yields several correlation times ranging from ps to μs for the motion of this segment of the phospholipid.[4,5]

The carbonyl region of phospholipids is likely to be critical for interactions with some peripheral proteins and other membrane components and might sense different motions than the phosphate portion of the molecule. In this report we present the first high-resolution ^{13}C field cycling relaxation studies of sn-2 carbonyl ^{13}C-labeled phosphatidylcholines (prepared by acylation of 1-palmitoyl-phosphatidyl-choline with either [1-^{13}C]-oleic acid or [1-^{13}C]-palmitic acid[7]) in small unilamellar vesicles (SUVs) as a direct probe of this interfacial region of phospholipids.

In small unilamellar vesicles (prepared by sonication) at 25°C, 1-palmitoyl-2-[1-^{13}C] oleoylphosphatidylchoine (PO[1-^{13}C]PC) mixed 1:1 with dioleoylphosphatidylmethanol (DOPMe) exhibits the field dependence profile shown in Figure 1A. Qualitatively, the profile between 0.04 and 11.7 T has features similar to that for ^{31}P R_1 of the POPC in these same bilayers.[4,5] The simplest analysis treats this field-dependent profile as the result of dipolar and CSA relaxation components characterized by a single correlation time τ_c, with a small contribution from a faster motion contributing CSA relaxation.[4] The balance of each of these terms for PO[1-^{13}C]PC in the POPC/DOPMe vesicles is shown in Figure 1B. Above 2 T, R_1 is dominated by CSA relaxation, while dipolar relaxation is the major mechanism below 1 T. The small rise in ^{13}C R_1 at high fields, due to faster, sub-nanosecond motions that contribute to CSA relaxation of the carbonyl, is much smaller for the ^{13}C site compared to the ^{31}P interaction.[4,5] More interestingly, the correlation time, τ_c, associated with the dipolar relaxation is 16±2 ns. This value is about 2-3 times the value obtained for the ^{31}P nucleus of POPC in POPC/DOPMe vesicles.[4,6] Thus, the 5-7 ns motion that effectively relaxes the phosphate group either does not alter the orientation of the sn-2 carbonyl site or contributes much less to relaxation of the ^{13}C-carbonyl compared to a slower motion. The value for $R_c(0)$, the relaxation rate extrapolated to zero field, from this region of the field dependence of R_1 provides an estimate of r_{CH}, the distance of the ^{13}C-labeled carbon to the major proton(s) that relax it,[4] in this case the sn-2 α-CH_2.[8] The value obtained, 2.4 Å, is similar to what one would expect for the ^{13}C-C-H distance involved.

We also examined the ^{13}C relaxation profile for an sn-2 ^{13}C-labeled saturated chain lipid, dipalmitoyl-PC (PP[1-^{13}C]PC), in this case mixed with unlabeled POPC to stabilize the small vesicles for extended observation at 40°C. The temperature was chosen to keep the fluid bilayer for the many acquisitions covering a course of a few days and to avoid getting too close to the maximum temperature of the shuttling system (50°C). As seen in Figure 2A, the PP[1-^{13}C]PC profile was very similar to that for the PO[1-^{13}C]PC and could be fit with a single τ_c of 16.2 ±2.5 ns; the average r_{CH} was 2.51 Å.

If the ^{13}C R_1 is measured at much lower fields (down to 0.002 T), there is a further rise in the ^{13}C relaxation rate that reflects the vesicle tumbling contribution to R_1.[5] For PO[1-^{13}C]PC mixed with DOPMe, the correlation time is 1.4±0.5 μs (Figure 2B). This is very close to what one would expect as the correlation time for rotation of 250-300 Å vesicles (the average diameter measured by dynamic light scattering for this preparation is 254 Å with 91% of the sizes between 200 and 400Å).[9] Geometric information can also be obtained from this very low field relaxation.[5] The area under this dipolar portion of the R_1 versus field curve compared to total dipolar relaxation yielded four values (±45.1° and ±65.7°) for the average θ_{CH} that the angle of the ^{13}C-H vector makes with respect to the bilayer normal. While the orientation of the carbonyl to α-CH_2 vectors in the crystal structure of dimyristoyl-PC might rule out the large negative angles,[10] there may be sufficient segmental motion of the CH_2 so that differentiating among these θ_{CH} is difficult. However, the ability to measure an averaged angle provides a novel way of characterizing changes induced by additives.

Cholesterol in a bilayer broadens the phase transition and makes membranes more gel-like. This physical change lengthens ^{31}P τ_c values dramatically[4,5]; it would also be expected to alter the dynamics of the sn-2 carbonyl group of PC. With 33 mol% cholesterol in the PO[1-^{13}C] PC/DOPMe vesicles (Figure 1A), the ^{13}C-carbonyl of PC shows an increase in τ_c with cholesterol, but it is relatively small – from 16±2 ns to 23±3 ns. For the same amount of cholesterol in the PP[1-^{13}C]PC/POPC vesicles, the τ_c increased to 28±4 ns (Figure 2A). Thus, the effect of cholesterol on the carbonyl motions of the two different vesicle systems is essentially the same – a small but significant increase in τ_c. For comparison, with cholesterol present, the ^{31}P τ_c increased from 5-7 ns to ~25 ns (data not shown). In the presence of the sterol, the nanosecond correlation times for both the carbonyl and phosphate moieties are essentially the same, whereas in the absence of cholesterol, the two nuclei are sensitive to motions on slightly different time scales.

Another way of looking at differences in the ^{31}P and ^{13}C motions in the nanosecond regime is to examine the temperature dependence of R_1 at a fixed low field where R_1 is directly proportional to τ_c. Different motions are likely to have different energetics. For the ^{13}C-labeled POPC, R_1 was measured at 0.06 T. An Arrhenius plot of the ^{13}C R_1 (Figure 3) leads to a slope of 27±7 kJ/mol. For comparison the temperature dependence for the ^{31}P of POPC at low field (0.032 T) yields a lower energy barrier, 12±3.8 kJ/mol.

These differences in the τ_c extracted from ^{31}P and ^{13}C in the same sample strongly indicate that the dipolar interactions of each group with its nearest protons do not reflect the same overall motion of the phospholipid. It has been suggested from molecular dynamics simulations that the ~10 ns ^{31}P τ_c arises from motions that treat the phospholipid as a cylinder encompassing the ^{31}P-glycerol-acyl chains that ‘wobbles’ around an axis perpendicular to the membrane surface.[6] The shorter τ_c for ^{31}P compared to ^{13}C suggests that the phosphorus motion also contains some faster local motion along with the motion supplied by wobble (presumably what dominates the more rigid carbonyl relaxation). Interestingly, both ^{31}P and ^{13}C have the same correlation time when cholesterol is present, suggesting the faster motion of the phosphorus has been dampened by the presence of the sterol. High resolution ^{13}C field cycling may also be useful for quantifying dynamics in other complex systems as long as the ^{13}C label (without attached protons) can be introduced.

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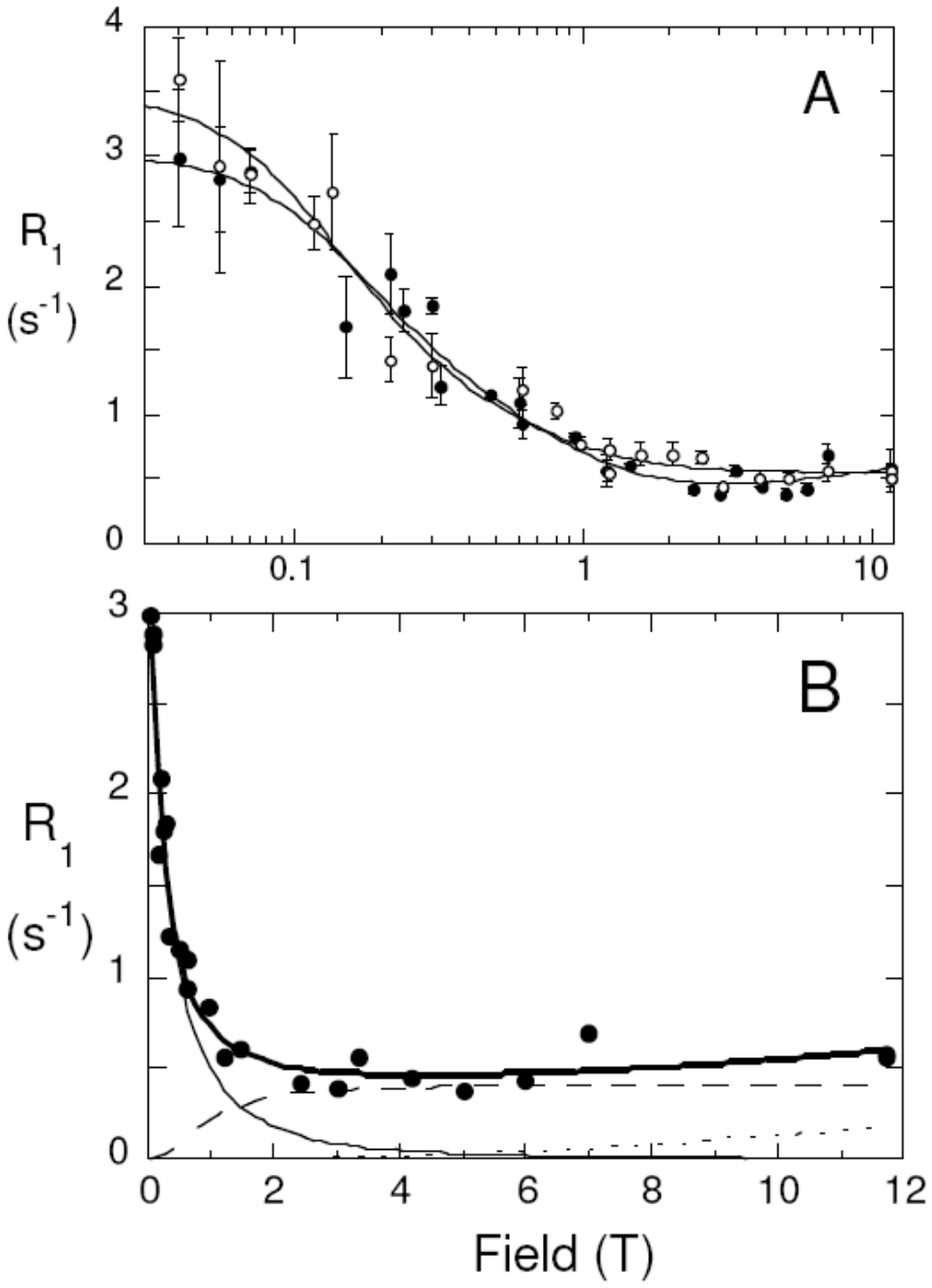

Figure 1.

(A) Field dependence of ^{13}C spin lattice relaxation rates, R_1, for PO[1-^{13}C]PC in SUVs composed of POPC (5 mM)/DOPMe (5 mM) in the absence (●) and presence (○) of 5 mM cholesterol; the semi-log plot emphasizes the behavior below 1T). (B) Deconvolution of R_1 in the absence of cholesterol into a dipolar (—) and CSA (- - -) component with correlation time τ_c, and a faster CSA motion (dotted line visible above 6T).

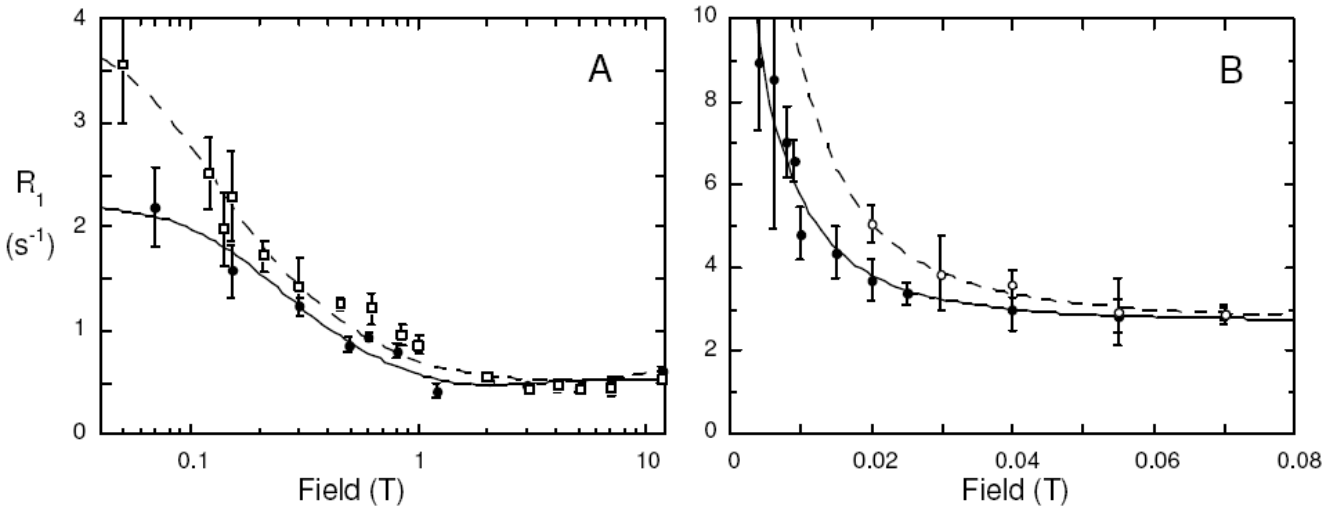

Figure 2.

(A) Field dependence of PP[1-^{13}C]PC in SUVs of DPPC (5 mM)/POPC (5 mM) at 40°C in the absence (●) and presence (□) of 5 mM cholesterol. (B) Very low field dependence of R_1 on field from 0.07 down to 0.004 T for PO[1-^{13}C]PC (mixed with DOPMe) in the absence (●) and presence (○) of 5 mM cholesterol.

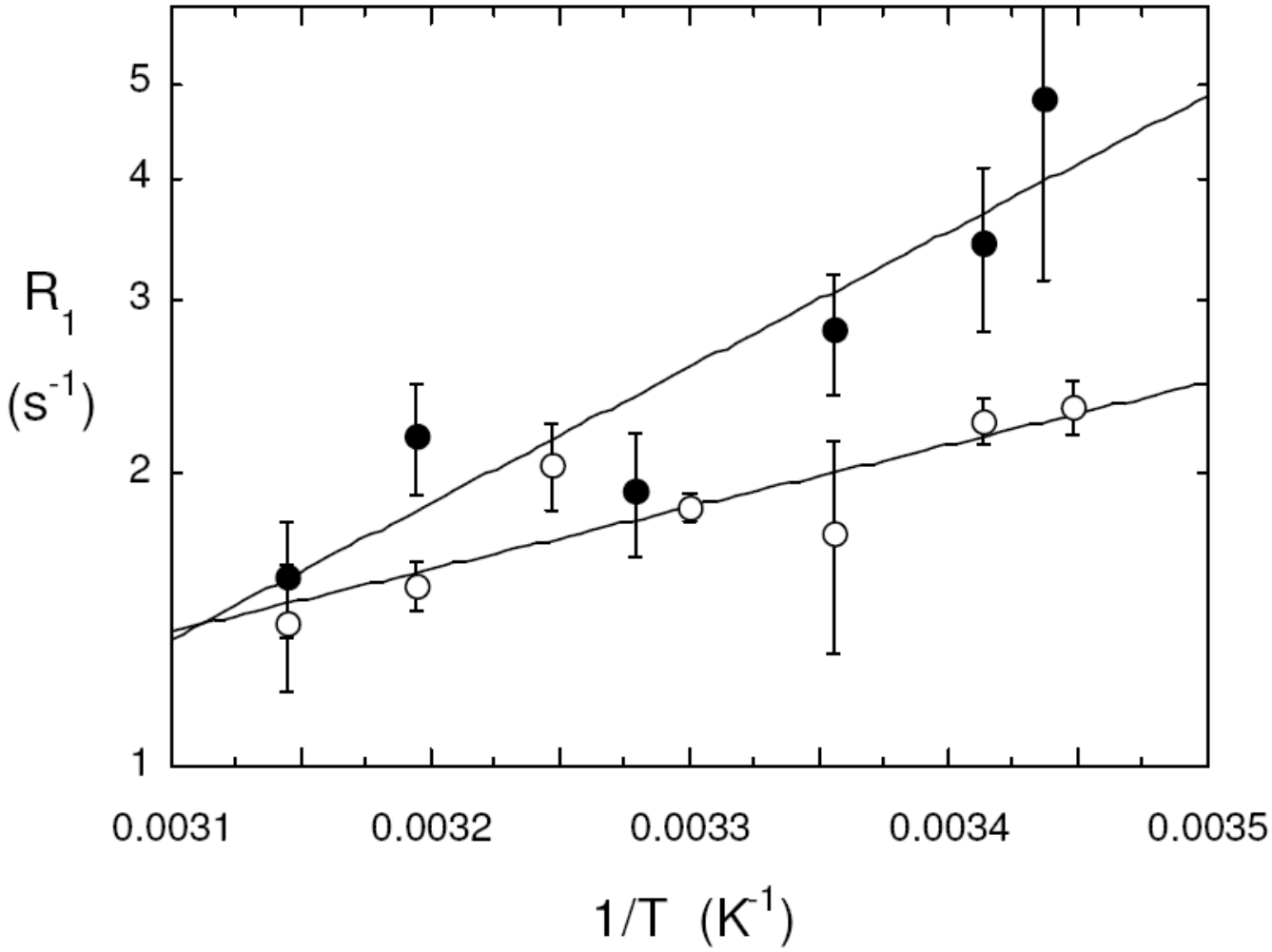

Figure 3.
Fixed low field spin lattice relaxation rates for POPC in small vesicles with DOPMe as a function of temperature: (●) ^{13}C R_1 for PO[1-^{13}C]PC measured at 0.06 T; (○) ^{31}P R_1 for POPC measured at 0.032 T.