GAMMA-GAMMA DIRECTIONAL CORRELATION STUDY OF MODEL MEMBRANES

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ABSTRACT Measurements of the perturbed gamma-gamma directional correlation were made for the 173-247 keV cascade in ^{111}Cd at the In ion site in dipalmitoylphosphatidylcholine liposomes. A time-dependent electric quadrupole interaction was observed. Below the order↔fluid phase transition temperature the exponential damping coefficient was $\lambda_2 = 0.033 \pm 0.007$ ns^{-1}. Above the transition temperature the coefficient was $\lambda_2 = 0.015 \pm 0.006$ ns^{-1} indicating a twofold increase in molecular mobility in the disordered phase. Absolute values for the motional correlation time, τ_c, were estimated to be on the order of a few nanoseconds. The presence of cholesterol was found to cause no significant difference in the molecular mobility of the phosphatidylcholine head groups in the ordered and fluid states.

INTRODUCTION

The existence of the order↔fluid phase transition in phospholipid vesicles or model membranes has been well established (Trauble, 1971; Fox, 1975; Overath and Thilo, 1978). The experimental techniques used in characterizing this transition include x-ray diffraction, differential scanning calorimetry, fluorescent intensity measurements, electron spin resonance, and nuclear magnetic resonance. Recently, the positron probe method has also proven to be useful in studying the phospholipid phase transition (McMahon et al., 1979).

Although all of these techniques have provided important information about vesicle phase transitions, they are limited in that they do not provide a direct measure of motional parameters at the molecular level. The gamma-gamma perturbed directional correlation (PDC) technique provides a method of obtaining direct information about molecular motion. This paper describes the first application of the PDC technique to an investigation of the order↔fluid phase transition in dipalmitoylphosphatidylcholine (DPPC) vesicles.

The theory of PDC has been reviewed extensively (Frauenfelder and Steffen, 1966), and applications to biophysical systems have been previously reported (Liepert et al., 1968; Meares et al., 1969; Graf et al., 1974; Richer et al., 1974). If a radioactive nuclide decays by a gamma-gamma cascade, we may define the directional correlation function, $W(\theta)$, as the relative probability that the second gamma photon is emitted at some angle θ with respect to the direction of emission of the first photon. First-order perturbation theory and the density matrix formalism have been utilized (Sakai et al., 1963) to obtain

$$W(\theta) = 1 + \sum_{k_{even}=2}^{\infty} A_{kk}(0)P_k(\cos\theta), \quad (1)$$

where the P_k (cos θ) are ordinary Legendre polynomials and the A_{kk} are weighing factors that depend on the spins of the excited, intermediate, and ground states, and on the multipolarities of the two photons. For nuclei that are generally used for the PDC technique, the coefficient $A_{44} \ll A_{22}$ and the correlation function becomes simply

$$W(\theta) = 1 + A_{22}(0)P_2(\cos\theta), \quad (2)$$

where again $A_{22}(0)$ refers to a nucleus that is not perturbed during its intermediate lifetime.

Information is easily obtained by measuring the anisotropy defined by

$$A(0) = \frac{W(\pi) - W(\pi/2)}{W(\pi/2)}, \quad (3)$$

which is related to $A_{22}(0)$ by

$$A_{22}(0) = \frac{2A(0)}{3 + A(0)} \quad (4)$$

after appropriate corrections are made for the finite size of the detectors and source (Yates, 1963; Rose, 1953).

In many cases, the intermediate level lifetime τ_N will be long enough that the intermediate state nucleus will have time to interact with any perturbing force fields that are present. The time-dependent correlation function then becomes (Frauenfelder and Steffen, 1966)

$$W(\theta, t) = 1 + A_{22}(0)\, G_{22}(t)\, P_2(\cos\theta). \quad (5)$$

The function $G_{22}(t)$, known as the perturbation factor, contains all the information about the interaction. It

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depends on the spin of the intermediate level and parameters involved in the particular kind of perturbation that occurs.

The isotope used in this study was ^{111}In; it decays by electron capture to ^{111}Cd, with a recoil energy of 2.1 eV, and then decays to its ground state by way of a 173-247 keV gamma-gamma cascade. The coefficient $A_{22}(0) = -0.18$ is known from work (Steffen, 1956) in which the correlation time of the nuclear environment is much less than the intermediate state lifetime ($T_{1/2}$ = 85 ns). For a static electric quadrupole interaction the perturbation factor (Frauenfelder and Steffen, 1966) is given by

$$G_{22}(t) = \frac{1}{5} + \frac{13}{35}\cos\omega_0 t + \frac{2}{7}\cos 2\omega_0 t + \frac{1}{7}\cos 3\omega_0 t, \quad (6)$$

where the interaction frequency, ω_0, is dependent on the product of the nuclear quadrupole moment, Q, and the strength of the electric field gradient, V_{zz}, at the probe nuclide site, and is given by

$$\omega_0 = \frac{3\pi e|Q\,V_{zz}|}{10\,h}, \quad (7)$$

where V_{zz} is assumed to be axially symmetric, e is the electronic charge, and h is Planck's constant.

If the nuclear environment is rapidly fluctuating, the perturbation factor is (Abragam and Pound, 1953),

$$G_{22}(t) = e^{-\lambda_2 t}, \quad (8)$$

where the damping coefficient, λ_2, is dependent on the product of the correlation time of the fluctuation, τ_c, and the mean-square value of the electric field gradient, and is given by

$$\lambda_2 = \frac{63}{250}\frac{\pi^2\tau_c}{h^2}(eQ)^2(\overline{V_{zz}^2}). \quad (9)$$

For slow molecular motion, as in the case of viscous liquids or with the nuclide attached to a macromolecule in aqueous solution, the perturbation factor (Marshall and Meares, 1972) becomes

$$G_{22}(t) = e^{-\lambda_2 t}\left(\sum_{n=0}^{3} S_n \cos n\omega_0 t\right), \quad (10)$$

where the above sum is defined in Eq. 6.

A determination of the perturbation factor thus yields information about the motion of the molecule to which the PDC nuclide is attached.

METHODS

Vesicles were prepared from synthetic DPPC (98% pure) and cholesterol (99% pure) as obtained commercially (Sigma Chemical Co., St. Louis, MO), without further purification. The samples were weighed and dissolved in a choloroform/methanol solution (9:1 vol/vol) in a small test tube. The solvent was removed by a dry nitrogen stream to leave the lipid as a thin film on the test tube walls. Complete removal of the solvent was insured by keeping the test tube under vacuum for 4 h. To the dry lipid, 2.5 mM HCl was added to yield a lipid concentration of 50 mg/ml. The tube was then sealed and the lipid was dispersed by mixing on a vortex mixer. The sample was sonicated in a bath-type sonicator (Laboratory Supplies Company, Inc. Hicksville, New York) at 48°C until the turbid suspension became clear, and then ~25 μCi of $^{111}InCl_3$ in 30–125 μl of 0.05 N HCl was added. The mixture was incubated at the same temperature for 20 min and the pH adjusted to 7.5 with 0.12 M NaOH. Finally, the vesicle suspension was transferred to a water-jacketed capillary for the PDC measurements.

Indium normally precipitates out of solution as a hydroxide at a pH >3 or 4. When the pH is raised in the presence of DPPC liposomes, the negatively charged phosphate portion of the phosphatidylcholine head group offers a strongly competitive electrostatic binding site to the positively charged indium ion. Verification of this binding was provided as follows: 1 ml of 1,000 ppm nonradioactive In was added to a vesicle preparation (100 mg lipid/ml); the resulting mixture was separated on a Sepharose 4B column, and all of the eluted In was found by atomic absorption spectroscopy to be contained in the vesicle fractions. The mole ratio of In to lipid for this binding test was ~1:15; however, for the ^{111}In labeled vesicles used in the PDC measurements, the mole ratio of In was ~1:10^8. Such a small amount of ^{111}In will not cause any significant perturbation on the phospholipid phase transition. The attachment of the In ion to the hydrocarbon chains is prevented by the fact that the electrostatic binding energy of the In head-group interaction is orders of magnitude larger than the hydrophobic interaction that would be required to localize the positively charged In ion inside the nonpolar region of the membrane.

The sonication of the DPPC lipid dispersion caused the formation of small unilamellar vesicles (SUV) that have a phase transition temperature of 35°C. Because SUV are unstable, they are expected to undergo a substantial recombination into multilamellar vesicles (MLV) during the course of the 3-d data collection period. This was evidenced by an increase in turbidity in the sample. Because MLV have a transition temperature of 41°C (Suurkuusk et al., 1976), water-bath temperatures of 23° and 48°C

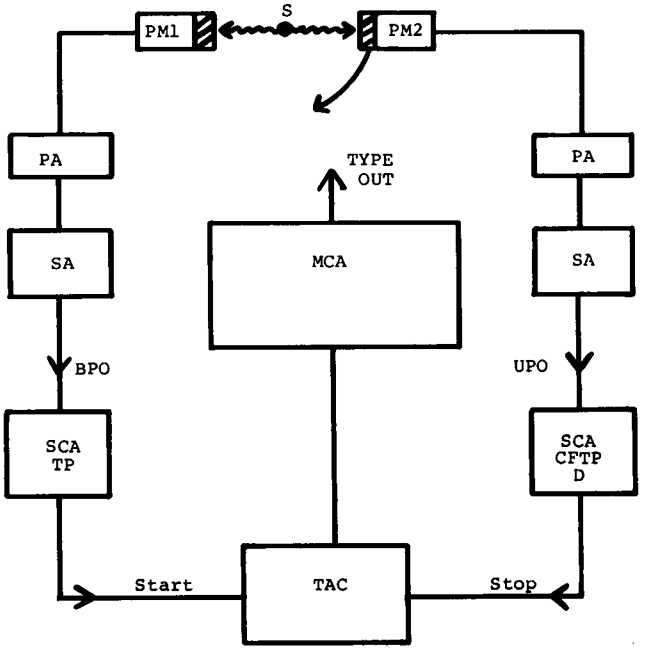

FIGURE 1 Schematic of the time differential coincidence spectrometer. PA, preamplifier; PM, photomultiplier; SA, shaping amplifier; TP, time pickoff; D, delay; CFTP, constant fraction time pickoff; SCA, single channel analyzer; TAC, time-to-amplitude converter; MCA, multichannel analyzer; BPO, bipolar output; UPO, unipolar output.

were used for the PDC measurements to insure that completely ordered and completely fluid states were realized.

The time differential coincidence spectrometer used for the PDC measurements is shown in Fig. 1. It is a two-detector system utilizing 1.5 × 1.5 in. NaI crystals. One detector is movable and may be locked into place at angles of 90° and 180° relative to the fixed detector.

The negative pulses from the photomultipliers were first shaped by the preamplifiers and then sent to the shaping amplifiers. The timing single-channel analyzer (TSCA) connected to the fixed detector amplifier puts out a negative timing pulse when its bipolar input pulse crosses over zero voltage ("crossover-pickoff" timing). On the variable detector side, the timing pulse is produced when the input pulse reaches 20% of its maximum pulse height ("constant fraction" timing).

The TSCA also act as pulse height discriminators. The energy windows of both detectors were set to cut of just below the 173 keV peak and just above the 247 keV photopeak. The ^{111}In decay is a very clean process, and the only photons that are being discriminated against are the Cd K-shell x rays. The 173- and 247-keV pulses would normally act as start and stop pulses, respectively, for the time-to-amplitude converter (TAC). Because both detectors were allowed to record both photons, the start and stop pulses interchanged roles, and a delay was inserted in one side of the coincidence circuit so that prompt coincidences ($t = 0$) corresponded to one-fourth maximum output pulses from the TAC. The resulting coincidence spectrum was then actually a double spectrum symmetric about the $t = 0$ axis. This technique essentially doubles the data collection rate. The 4-μs nominal time span used for the experiments made it possible to determine the random coincidence rate from data near the end of the spectrum.

RESULTS

The perturbation factors for the 173-247 keV gamma-gamma cascade of ^{111}Cd bound to the DPPC bilayer below and above the phase transition temperature are shown in Figs. 2 and 3, respectively. The theoretical curves result from a least-squares fit to a perturbation factor of the form previously used by Martin and Skov (1978),

$$G_{22}(t)_{TH} = A + B\,G_{22}(t). \tag{11}$$

The analysis was corrected for the time resolution of the system, and, in every case, gave the best fit for a $G_{22}(t)$ of the form given in Eq. 8. The resulting values of λ_2 are 0.033 ± 0.007 ns^{-1} at 23°C, and 0.015 ± 0.006 ns^{-1} at 48°C.

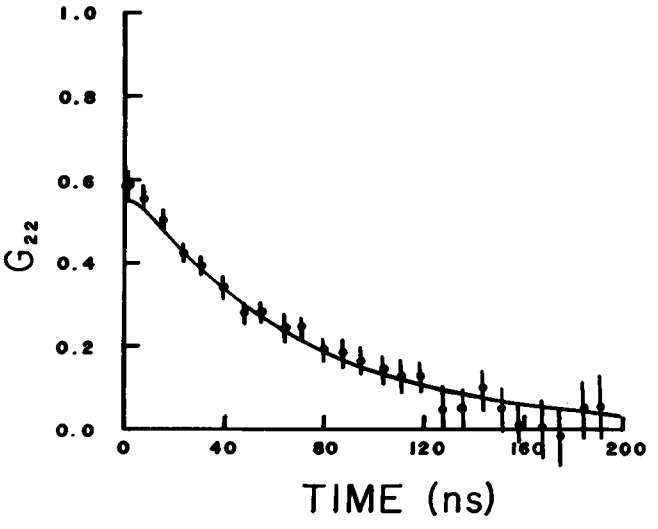

FIGURE 3 Perturbation factor in DPPC (50 mg/ml) at 48°C. A least-squares analysis gives $\lambda_2 = 0.015 \pm 0.006$ ns^{-1}.

The effect of cholesterol was investigated by ^{111}In labeling of vesicles composed of 15 mol % cholesterol and 85 mol % DPPC. The perturbation factors below and above T_c are shown in Figs. 4 and 5, respectively. In this case the values of λ_2 are very similar at 0.019 ± 0.005 ns^{-1} below the phase transition and 0.023 ± 0.008 ns^{-1} above.

From Eq. 9, the motional correlation time, τ_c, is proportional to λ_2. The results thus indicate a twofold decrease in the correlation time of the DPPC molecule in passing from the ordered state to the fluid state. The presence of cholesterol practically eliminates this change.

The electric field gradient for an ionic bond such as is present here is generally on the order of 10^{20} V/m^2. Using

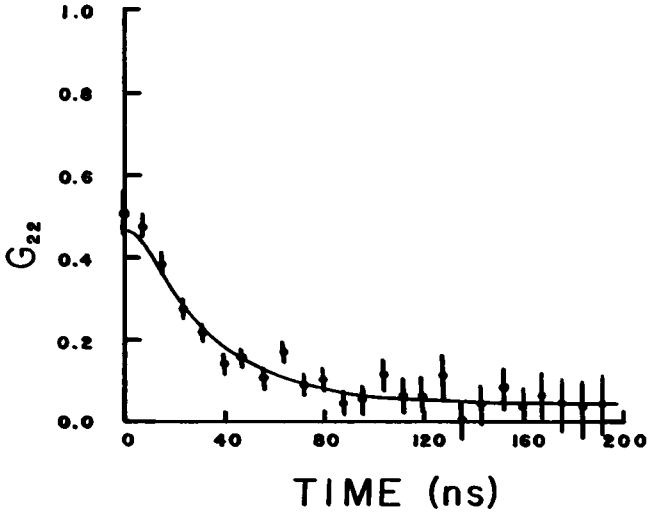

FIGURE 2 Perturbation factor in DPPC (50 mg/ml) at 23°C. A least-squares analysis gives $\lambda_2 = 0.033 \pm 0.007$ ns^{-1}.

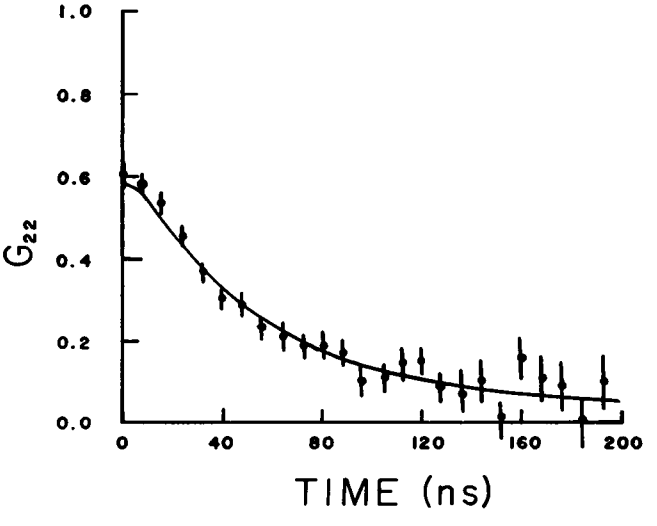

FIGURE 4 Perturbation factor in DPPC-15 mol % cholesterol (50 mg/ml) at 23°C. A least-squares analysis gives $\lambda_2 = 0.019 \pm 0.005$ ns^{-1}.

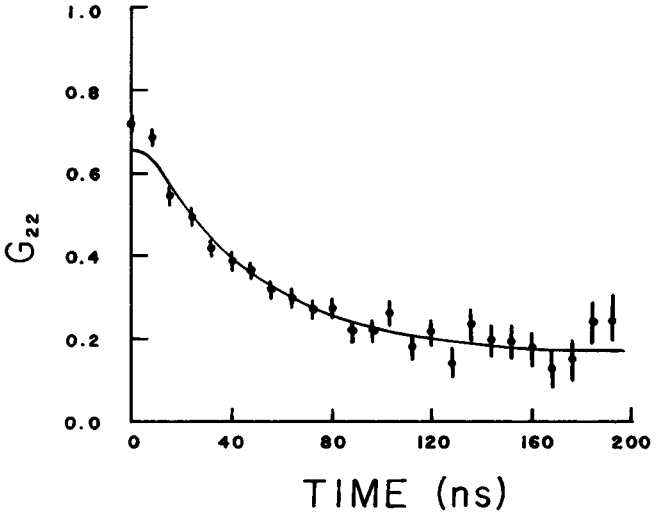

FIGURE 5 Perturbation factor in DPPC-15 mol % cholesterol (50 mg/ml) at 48°C. A least-squares analysis gives $\lambda_2 = 0.023 \pm 0.008$ ns^{-1}.

this value and the values of λ_2 in Eq. 9 gives order of magnitude values for τ_c. The resulting values range from 1.5 to 3.3 ns.

DISCUSSION

The above statement that τ_c is proportional to λ_2 assumes that the root-mean-square value of the electric field gradient remains fairly constant through the phase transition. This is a reasonable assumption because the PDC nuclide is bound to the DPPC head group, whereas the phase transition consists of the disruption of van der Waals interactions between the carbon chains. There is, therefore, no change expected in the average chemical environment of the ^{111}Cd nuclide due to the phase transition. The small recoil energy of the electron capture process (2.1 eV) will insure that the probe nuclide is not dislodged from its binding site, and the relatively high electron density of the charged head groups will prevent any time-dependent electron shell effects by rapidly filling the electron hole. The correlation time of the fluctuating electric field gradient then reflects the increased molecular motion in the disordered state. For pure DPPC, the motional correlation time (i.e., period of motion) in the disordered state is one-half of the corresponding value for the ordered state, thus indicating a twofold increase in the rate of molecular motion or fluidity. The term molecular motion is used here to refer to a combination of translation and rotation of the head group, which is naturally influenced by the order or disorder of the hydrophobic chains.

Positron and differential scanning calorimetry measurements have previously shown a broadening of the temperature range of the phase transition and a lowering of the transition enthalpy due to the presence of cholesterol (McMahon et al., 1979). The PDC results reported here indicate no significant differences in the molecular motion above the phase transition as compared to below the transition. This result is consistent with NMR results (Stockton and Smith, 1976) which indicate that the cholesterol molecule creates an ordered state from the polar head group inward to approximately carbon number 10–12. The PDC nuclide, being attached to the head group, would be expected to experience very little change in its motional state when the phase transition is limited to the far ends of the carbon chains.

X-ray diffraction analysis is capable of distinguishing the fluid phospholipid diffraction spacing (4.6 Å) from the phospholipid spacing in the ordered state (4.2 Å). Studies done on biological membranes (Wilkins et al., 1971) have shown an enhancement of the 4.2-Å spacing due to the presence of cholesterol. All of these results are therefore consistent with tighter phospholipid packing and higher immobilization due to cholesterol, particularly for the portions of the hydrocarbon chains nearest the head groups.

The presence of the cholesterol near the ^{111}Cd site may influence the chemical environment there, and therefore change the electric field gradient. It is thus not safe to compare the values of λ_2 for pure DPPC to those for the DPPC/cholesterol system and expect an accurate determination of the relative motion.

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