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Rapid report

Effects of cholesterol and temperature on the permeability of dimyristoylphosphatidylcholine bilayers near the chain melting phase transition

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Abstract

The passive leakage of glucose across bilayers of dimyristoylphosphatidylcholine (DMPC), cholesterol (variable), and dicetyl phosphate (constant 5.9 mol%) has been measured as efflux over 30 min from multilamellar vesicles. Bilayer cholesterol was varied from 20 mol% to 40 mol%. Glucose permeation rates were measured from 10°C to 36°C, and showed a maximum in permeability at 24°C, the DMPC phase transition temperature. Increasing the bilayer cholesterol content above 20 mol% reduced that permeability peak. These results are quite consistent with a large number of similar bilayer permeability studies over the past 25 years. However, they are not consistent with a previous study of these same systems, which reported increased glucose permeability with temperature, without any maximum at or near the lipid chain melting temperature (K. Inoue, Biochim. Biophys. Acta 339 (1974) 390–402).

Keywords: Phosphatidylcholine; Multilamellar vesicle; Cholesterol; Glucose; Permeability; Phase transition

It is well known that phospholipid bilayers can exhibit dramatic changes in permeability to aqueous solutes near the gel–fluid (chain melting) phase transition [1–7]. Details of the permeability changes depend, sometimes strongly, on the solute permeants, the lipids and other components of the bilayer, and the type of vesicle samples used [1–7]. In almost all cases, if the phospholipid bilayer undergoes a gel–fluid phase transition, the bilayer permeability to both charged and polar aqueous solutes exhibits a maximum near the phase transition temperature (T_m). An exception has been reported recently by Langner and Hui [7], who show significantly different effects on bilayer excess heat capacity and permeability for the addition of 5 and 10 mol% stearic acid vs. oleic acid to unilamellar vesicle preparations of dimyristoylphosphatidylcholine (DMPC). Another exception is from Inoue, who reported no maximum, but instead a monotonic increase with temperature in glucose permeability of multilamellar vesicles of DMPC and dipalmitoylphosphatidylcholine (DPPC) containing various amounts of cholesterol [8].

Several suggested mechanisms for a permeability maximum near the lipid phase transition have focused on packing defects in the boundary lipids between co-existing gel and fluid domains in the bilayer [1,3,9]. We and others have suggested [2,10–12] that

Abbreviations: DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DPPC, 1,2-palmitoyl-*sn*-glycero-3-phosphocholine; MLV, multilamellar vesicles; T_m, phase transition temperature; NADPH, reduced nicotinamide adenine dinucleotide phosphate

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increases in permeation and other kinetic effects observed near bilayer phase transitions are due to transient bilayer defects caused by the dramatic lateral area (and volume) changes [10,14] produced by lipid domains as they fluctuate between the gel and fluid states in dynamic equilibrium. Both mechanisms may in fact contribute to the increase in permeability near the phase transition [12]. Fluctuations in area and volume (and hence density) lead to an increased lateral compressibility of the bilayer over that of either the gel or fluid state. Several approaches for quantifying passive permeability of bilayers in terms of the enhanced compressibility near T_m have been reported [4,12–17].

Both experimental and theoretical studies have suggested that, although the bilayer gel–fluid transition is substantially first order, some may be near a higher order phase transition [11–13]. Such higher order or continuous phase transitions can occur at a critical point, well known in liquid–vapor phase equilibria, and in magnetic systems. A critical point is the terminus of a two phase coexistence boundary line on the pressure–temperature phase diagram, below which the line maps the two phase coexistence, and above which there is no more phase boundary, hence no phase transition. A bilayer near a critical point would be expected to exhibit fluctuations on all length scales, causing dramatic effects in order parameters and divergent tendencies in response functions such as specific heat and compressibility.

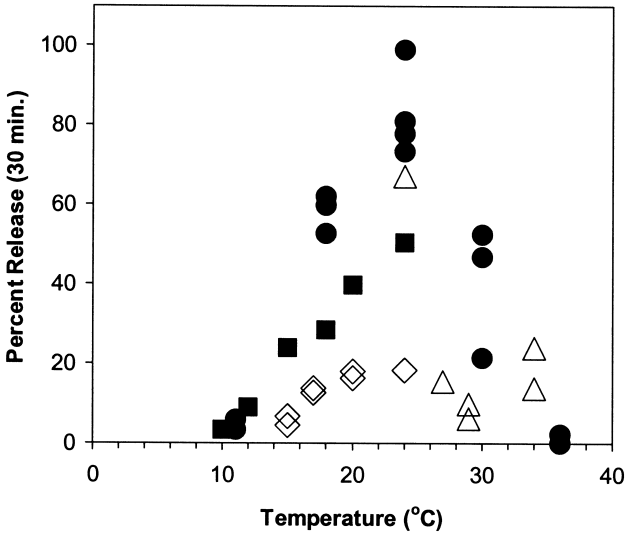

Fig. 1. Percent release over 30 min of trapped glucose from DMPC MLV, made with 20 mol% cholesterol and 5.9 mol% dicetyl phosphate, at various incubation temperatures. ■,◇, vesicle batches dialyzed below 6°C and warmed to incubation temperature; ●, vesicle batches dialyzed below 6°C and used throughout the temperature range; △, vesicle batches dialyzed above 34°C and cooled to incubation temperature.

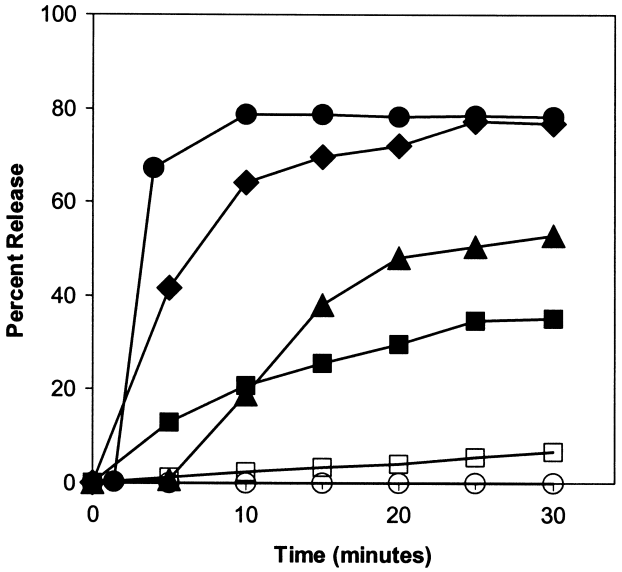

Fig. 2. Percent of trapped glucose released as a function of time from DMPC vesicles with 20 mol% cholesterol and 5.9 mol% dicetyl phosphate, while incubated at temperatures of 11°C (□), 18°C (▲), 24°C (dialyzed at 6°C) (●), 24°C (dialyzed at 36°C) (◆), 30°C (■), and 36°C (○).

Reliable experimental data for solute permeability of model and biological membranes are essential for advancing the understanding of the structure and function of all membranes. The dramatic anomaly of the missing permeability maximum [8] is problematic for both theoretical and experimental progress. Along with the theoretical efforts cited above, applied research studies are underway to examine the possibilities for using vesicles as drug delivery agents, with selective permeability as a potential means for targeting the encapsulated drug for the desired tissue [18–20].

In this study, the passive permeability of glucose through fully hydrated DMPC bilayers containing dicetyl phosphate and cholesterol has been measured over the temperature range 10–36°C. Dicetyl phosphate, used at low concentration to improve bilayer stability without noticeable effect on the DMPC

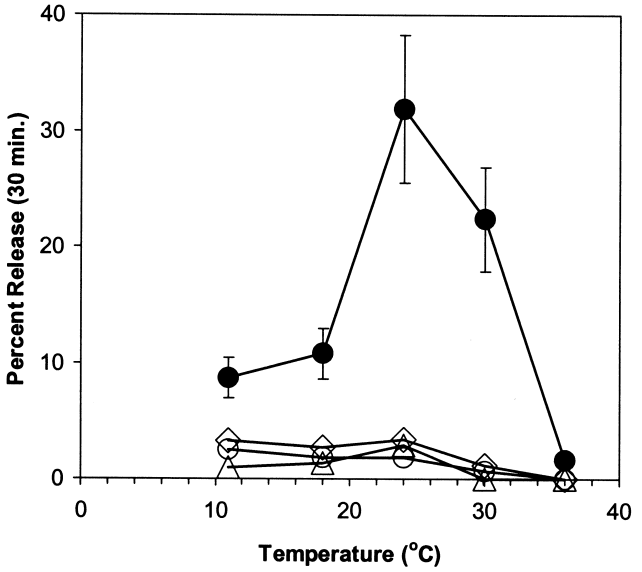

Fig. 3. Average percent release of trapped glucose from DMPC MLV containing cholesterol at 25 mol% (●), 30 mol% (◇), 35 mol% (△), and 40 mol% (○) over a 30 min period as a function of temperature. The 25 mol% data are averages of several samples, either heated from 6°C or cooled from 36°C, as in Fig. 1. Error bars represent the total spread of measurements.

chain melting transition, was held constant at 5.9 mol%, and cholesterol levels varied from 20 mol% to 40 mol%, allowing comparison with previous reports [8].

DMPC was purchased from Avanti Polar Lipids (Alabaster, AL), cholesterol, D-glucose, dicetyl phosphate and enzymatic glucose assay reagent were purchased from Sigma (St. Louis, MO). The purity of lipids and cholesterol was verified by chromatography. Bilayer constituents in proper ratios were dissolved in chloroform and dried to a uniform thin film under reduced pressure by rotary evaporation, then desiccated under vacuum for at least 4 h to remove any remaining solvent. Enough 0.3 M aqueous glucose at 40°C was added to the lipid film to yield 9.30 nmoles per μl of suspension, then vortex-agitated 2 or more minutes above 35°C, producing a uniform suspension of multilamellar vesicles (MLV). To remove the external glucose, vesicle suspensions were dialyzed against 0.187 M aqueous NaCl solution, isosmotic with 0.3 M glucose. Vesicles with less than 30 mol% cholesterol were dialyzed at 6°C for at least 8 h, or near 36°C for at least 3 h to exchange the free glucose for NaCl without appreciable vesicle leakage or osmotic swelling. Vesicles with 30 mol% or more cholesterol contained glucose well at all temperatures, and thus were dialyzed at room temperature for at least 4 h.

Glucose efflux from vesicles was determined by measuring the optical absorbance change with the reduction of nicotinamide adenine dinucleotide phosphate (to NADPH), produced by a hexokinase/glucose-6-phosphate dehydrogenase assay reagent (Sigma, used at 0.75 of the suggested concentration). The glucose assay was carried out in 1 cm path length cuvettes, where the absorbance was measured at 340 nm in a Spectronic 700 spectrophotometer. Glucose efflux from the MLV, rather than enzyme influx, determined the extent of the assay reaction due to the size differences of those solute reactants. Sample temperature was held constant by circulating thermostatic water through the four-compartment cuvette holder; the sample temperature was monitored with a thermistor in one of the cuvettes. Duplicate experiments were run in parallel in the other three cuvettes, in one of which the absorbance was recorded as a function of time. A 10 μl aliquot of

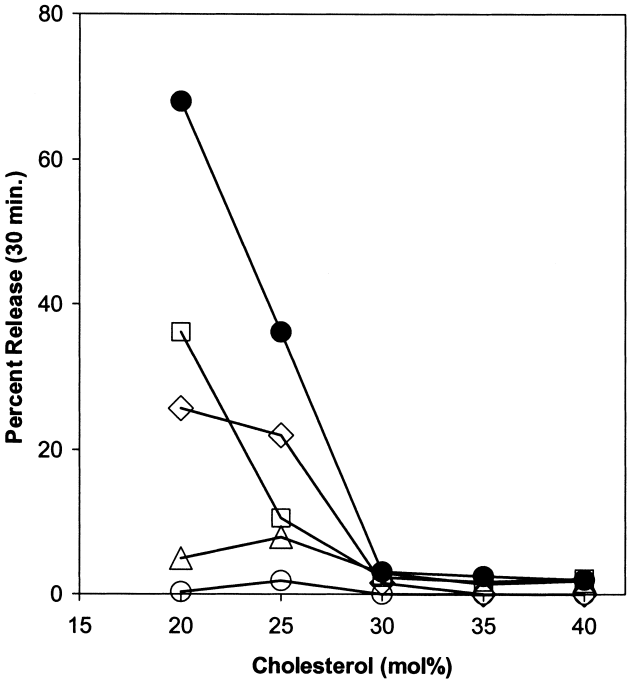

Fig. 4. Average percent release of trapped glucose from DMPC MLV over 30 min as a function of bilayer cholesterol content. Incubation temperatures: 11°C (△); 18°C (□); 24°C (●); 30°C (◇); 36°C (○).

dialyzed MLV suspension was added to a cuvette containing 2 ml of the glucose assay reagent. The initial absorbance at 340 nm was recorded after 5–10 min to measure any untrapped glucose remaining after dialysis. This initial 5–10 min untrapped glucose assay was carried out at temperatures either below 10°C, or above 34°C, for MLV with less than 30 mol% cholesterol, and at the permeability incubation temperature for all other preparations. After this initial untrapped glucose assay, the cuvettes with vesicle suspensions were brought to the desired temperature in the thermostatic sample chamber and incubated. For comparison with other reports, absorbance at 340 nm (assayed glucose efflux) was recorded every 5 min for 30 min total incubation, sufficient time to insure completion of the assay reaction. Samples were then brought to room temperature and the vesicles were lysed by the addition of 5 μl of Triton X-100 in chloroform (at least 50% Triton by volume). Absorbance at 340 nm was measured after 5 min, giving a measure of the total glucose initially trapped in the MLV, thus allowing calculation of the percent of trapped glucose released during the 30 min thermostatic incubation. On several occasions, additional Triton X-100 in chloroform was added to the cuvette, at least doubling the amount added, with no significant additional absorbance observed.

In order to measure bilayer glucose permeability for the same MLV preparation throughout the temperature range, in some experiments a 10 μl sample of vesicles dialyzed and stored at 6°C was added to a glucose assay held at 36°C and used for the same measurements as those dialyzed at 36°C, to determine leakage rates both below and above 24°C, the lipid T_m for DMPC MLV [22]. Vesicles with 20 mol% cholesterol lost as much as half of their trapped glucose when quickly transferred through T_m by this method. This glucose leakage was evident in measurements showing both increased exterior glucose and lower vesicle containment values. Little or no glucose was lost upon quickly transferring aliquots of MLV with cholesterol levels above 25 mol% through the lipid T_m.

Vesicles with 20 mol% cholesterol exhibited increased glucose release as the incubation temperatures were raised, approaching T_m from lower temperatures (Fig. 1). Values near 100% release were sometimes obtained in a 30 min incubation period. These vesicles also showed increased percent glucose release as the incubation temperatures were lowered, approaching T_m from higher temperatures, thus showing a maximum in bilayer permeability near the lipid T_m (Fig. 1).

Not surprisingly, the largest spread in data points due to different MLV preparations occurred with the 20 mol% cholesterol vesicles (Fig. 1), those most prone to leakage under any conditions. Results with vesicle samples from the same preparation were self-consistent across temperature (Fig. 1). Some preparations of 20 mol% cholesterol trapped and/or contained insufficient glucose for further measurements, and those that did contain sufficient glucose for these experiments varied in the amount trapped. Others have experienced similar variations while determining ion permeability among different preparations [1]. Optical absorbance values, measured after lysing the 20 mol% cholesterol vesicles, varied from approx. 0.1 to 0.5, under the previously described assay conditions. Smaller absorbance values due to incubated leakage were measured in those samples referred to earlier that were transferred through T_m prior to the permeability measurements; the data obtained with these samples correspond to filled circles at and above 24°C in Fig. 1.

In addition to the variation among preparations, the addition of Triton X-100 in chloroform used to lyse the bilayers sometimes had a small but random effect on the apparent absorbance of the vesicle sample, due to light scattering. These two variations are largely responsible for the spread in the 20 mol% cholesterol data seen in Fig. 1.

The apparent kinetics of glucose leakage from the various MLV preparations was consistent over the variety of samples. The shapes of the curves from 0 to 30 min were all relatively similar to those in Fig. 2, showing the assayed glucose leakage over time for 20 mol% cholesterol vesicles that were incubated at different temperatures. The same general shape of the temperature profile showing a permeability maximum (Fig. 1) would be obtained for any leakage time interval chosen for comparison (Fig. 2).

In cases where the cuvette and contents were brought to incubation temperature from below 10°C, a time lag was evident (Fig. 2). This most likely corresponds to the time necessary for the cu-

vette and contents to reach a temperature sufficient for leakage to begin. This time lag is not evident in samples cooled to incubation temperature from 36°C. Convenience dictated that samples incubated at 36°C to assay untrapped glucose were transferred via pipet to a cuvette already at the incubation temperature. Thus, these samples reached incubation temperatures more rapidly than the others.

Vesicles with 25 mol% cholesterol also exhibited maximum release at 24°C (Fig. 3). The highest value observed was 38%, a substantial decrease from the maximum value near 100% for some of the 20 mol% cholesterol MLV. Values for percent glucose release at T_m were obtained by approaching T_m from both higher and lower temperatures with some samples from the same MLV preparation, thus giving multiple sets of data for the 25 mol% cholesterol samples at 24°C. Vesicles with 30 mol% or more cholesterol exhibited relatively little glucose leakage at any temperature (Fig. 3).

The effect of bilayer cholesterol content on the passive release of trapped glucose from these MLV is summarized in Fig. 4, where the average percent glucose released at various temperatures is plotted as a function of cholesterol content. The enhanced release in the vicinity of 24°C, and the relatively low release at temperatures approaching 10°C or 36°C for 20 and 25 mol% cholesterol MLV, show why dialysis for these samples was carried out at the specified temperatures instead of room temperature. That bilayers with 30 mol% and more cholesterol required little attention to dialysis and storage temperatures is also evident from the data. One should not extrapolate this permeability effect of bilayer cholesterol too far; Corvera et al. have shown that at low enough levels, cholesterol increases solute permeability [17].

The findings of this study are in agreement with most studies of bilayer permeability to a wide variety of ionic and polar aqueous solutes [1–7]. Therefore, our results for bilayer permeability to glucose do not agree with the results reported by Inoue [8] for any of the similar preparations examined here. Vesicle preparations that exhibit the highly cooperative gel–fluid phase transition also exhibit a relatively sharp maximum in aqueous solute permeability at or near the phase transition temperature. This pattern persists in multicomponent bilayers made with cholesterol as well as mixtures of different lipids [1,2,6,7]. The correlation between permeability maxima and phase transitions in lipid bilayers is so strong that some have suggested using solute permeability to deduce bilayer phase diagrams [23]. The maximal rate of solute efflux near T_m at 20 mol% cholesterol decreases to the low levels characteristic of the gel or fluid phase at cholesterol levels near 30 mol% and greater, no doubt due to the decrease and elimination of the cooperative gel–fluid phase transition at those same cholesterol levels [22,24]. At temperatures sufficiently above and below T_m (e.g. $T_m \pm 12$°C in Figs. 1 and 4), there is relatively little efflux of solute at any of the measured cholesterol levels. The bilayer structural rearrangements and fluctuations occurring in the thermotropic lipid chain melting transition are thus most likely responsible for the unusual temperature dependent kinetics of passive bilayer permeability. Similar kinetic profiles with temperature that show a clear maximum near the lipid T_m, and thus can be rationalized with transient bilayer fluctuations, include phospholipase A_2 activity [25,26], protein incorporation into lipid bilayers [21], as well as other bilayer kinetic processes [27].

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