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Systematic Relationship between Phospholipase A_2 Activity and Dynamic Lipid Bilayer Microheterogeneity†

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ABSTRACT: A standing hypothesis in membrane biology implies that the collective physical properties of the lipid bilayer component of biological membranes can modulate the activity of membrane-associated proteins. We provide strong support for this hypothesis by exploring a model system, phospholipase A_2 catalyzed hydrolysis of one-component phospholipid vesicles. For vesicles of lipids with different chain lengths we observe, as a function of temperature and chain length, a systematic variation of the characteristic lag time for the onset of rapid phospholipase A_2 hydrolysis. These results, combined with theoretical results obtained from computer simulation of the gel-to-fluid phase transition in the unhydrolyzed lipid bilayers, enable us to demonstrate a strong correlation between the lag time and the degree of bilayer microheterogeneity in the phase transition region. Insight into the nature of this correlation suggests rational ways of modulating enzyme activity by modifying the physical properties of the lipid bilayer.

Phospholipase A_2 (PLA_2)[1] is a ubiquitous enzyme which catalyzes the hydrolysis of phospholipids to 1-acyllysophospholipids and fatty acids (Waite, 1991). The regulation of PLA_2 activity is of great importance for the understanding of many physiological processes, such as immunological response. Moreover, the functioning of PLA_2 at lipid−water interfaces of lipid aggregates lends itself, as a biophysical model system, to the study of generic effects involved in protein−membrane interactions (Burack & Biltonen, 1994; Sackmann, 1994; Mouritsen & Jørgensen, 1992). The process involves in the simplest case an initial binding of the enzyme to the lipid−water interface (Berg et al., 1991; Maloney et al., 1995; Scott et al., 1994) followed by the catalytic process (Waite, 1991). Under a wide range of experimental conditions, the hydrolysis time course exhibits a lag phase of a characteristic duration, τ, before a regime of rapid hydrolysis is entered (Apitz-Castro et al., 1982; Burack & Biltonen, 1994). We here examine the PLA_2 hydrolysis reaction of one-component, large unilamellar vesicles (LUV) of three different homodiacylphosphatidylcholines: dimyristoylphosphatidylcholine ($DC_{14}PC$), dipalmitoylphosphatidylcholine ($DC_{16}PC$), and distearoylphosphatidylcholine ($DC_{18}PC$). Despite the fact that the details of the apparent activation of PLA_2 at the end of the lag period are not fully understood (Burack & Biltonen, 1994; Hønger et al., 1995), the simplicity of our experimental approach allows us to correlate the characteristic time τ with the collective physical properties of the unhydrolyzed lipid

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[1] Abbreviations: PLA_2, phospholipase A_2; LUV, large unilamellar vesicles; DC_nPC, a homodiacylphosphatidylcholine with n carbon atoms in each acyl chain; $DC_{14}PC$, dimyristoylphosphatidylcholine; $DC_{16}PC$, dipalmitoylphosphatidylcholine; $DC_{18}PC$, distearoylphosphatidylcholine; τ, the characteristic lag time until rapid PLA_2 hydrolysis; T_m, the gel-to-fluid phase transition temperature.

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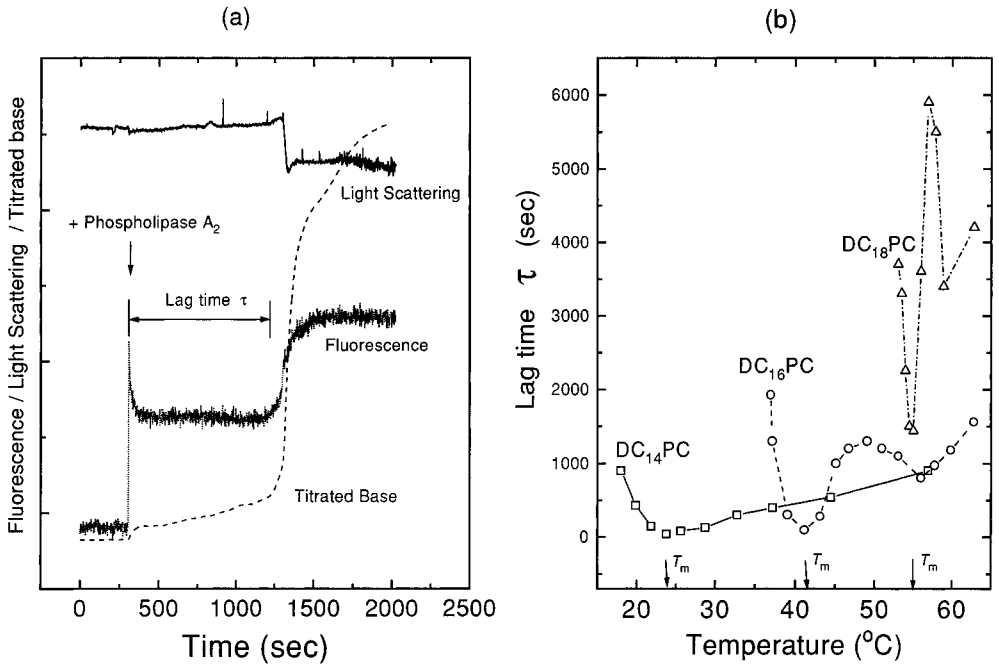

FIGURE 1: Phospholipase A_2 lag-time measurements. (a) Typical reaction time course for PLA_2-catalyzed hydrolysis of homodiacylphosphatidylcholine (DC_nPC) provided as one-component, 100 nm large unilamellar vesicles (LUV). The curves represent a hydrolysis reaction time course obtained for LUV of $DC_{16}PC$ at 39 °C. The PLA_2 hydrolysis reaction is monitored by pH-stat titration, intrinsic fluorescence from PLA_2 emitted at 340 nm upon excitation at 285 nm, and 90° light scattering from the suspension at 285 nm (all data are given in arbitrary units). The reaction is initiated at time 300 s by adding PLA_2 to the equilibrated vesicle suspension. The above observables report drastic changes in the reaction course after a characteristic lag time, τ, here defined by the onset in the increase in the intrinsic PLA_2 fluorescence intensity. (b) Lag time, τ, as a function of the reaction temperature for the hydrolysis of LUV of $DC_{14}PC$, $DC_{16}PC$, and $DC_{18}PC$. The lag times were reproducible within 10%.

bilayer, specifically the degree of microheterogeneity, which can be systematically varied by altering either a thermodynamic condition (e.g., the temperature) or a material property (e.g., the lipid chain length). In our study we take advantage of the fact that this microheterogeneity varies particularly strongly in the vicinity of the gel-to-fluid phase transition of the lipid bilayer (Bloom et al., 1991). Here we shall extract information about the microheterogeneity of the unhydrolyzed lipid bilayers from computer simulations on a microscopic model of the gel-to-fluid phase transition of the lipid bilayer (Mouritsen & Jørgensen, 1994).

MATERIALS AND METHODS

$DC_{14}PC$, $DC_{16}PC$, and $DC_{18}PC$ were obtained in powder form from Avanti Polar Lipids (Birmingham, AL) and used without further purification. One-component, 100 nm LUV were prepared as described in Hope et al. (1985). PLA_2 was purified from venom of *Agkistrodon piscivorus piscivorus* obtained from Sigma Chemical Co. (St. Louis, MO) (Maraganore et al., 1984). The PLA_2 hydrolysis reaction is monitored by pH-stat titration, intrinsic fluorescence from PLA_2 emitted at 340 nm upon excitation at 285 nm, and 90° light scattering from the suspension at 285 nm. Fluorescence and scattering data were recorded using a SLM DMX-1100 spectrofluorometer. pH-stat titration was performed simultaneously with an ABU80 autoburet from Radiometer [see e.g., Burack et al. (1993)]. All data in Figure 1b were produced under the following conditions: 150 μM DC_nPC LUV, 150 nM PLA_2, 150 mM KCl, 1 mM NaN_3, 30 μM $CaCl_2$, 10 μM EDTA, and 10 mM Hepes (pH 8). The vesicle suspensions were equilibrated for 30 min prior to addition of PLA_2.

Monte Carlo computer simulation studies of the three different lipid bilayers of $DC_{14}PC$, $DC_{16}PC$, and $DC_{18}PC$ were performed on the basis of a statistical mechanical model of the gel-to-fluid chain melting transition of one-component lipid bilayers [described in Mouritsen and Jørgensen (1994)]. This model, which accounts for the conformational statistics of the acyl chains, includes several terms which in a detailed way describe, e.g., the intrachain conformations and the van der Waals interactions between the various acyl chain conformations.

The Monte Carlo computer simulation results reported were obtained on systems corresponding to 5000 lipid molecules.

RESULTS AND DISCUSSION

Figure 1a represents a typical PLA_2 reaction time course for $DC_{16}PC$ vesicles. As indicated in Figure 1a, the time τ marks a critical transition in the rate of hydrolysis as monitored by pH-stat titration. Other observables indicate major changes in the reaction conditions at this stage (Burack & Biltonen, 1994; Hønger et al., 1995), e.g., strong variations in intrinsic PLA_2 tryptophan fluorescence intensity and changes in lipid morphology, here monitored by 90° light scattering. We shall adopt the commonly used definition of τ based on the rapid change in the fluorescence intensity (Berg et al., 1991; Burack & Biltonen, 1994). Figure 1b shows the resulting τ as a function of temperature for LUV of $DC_{14}PC$, $DC_{16}PC$, and $DC_{18}PC$ under identical reaction conditions. The three curves of $\tau(T)$ each display a minimum at the respective gel-to-fluid phase transition temperature, T_m. This minimum is deeper and wider the shorter the lipid chain is; cf. Figure 2a. For $DC_{16}PC$ and $DC_{18}PC$, $\tau(T)$ furthermore exhibits a local maximum above

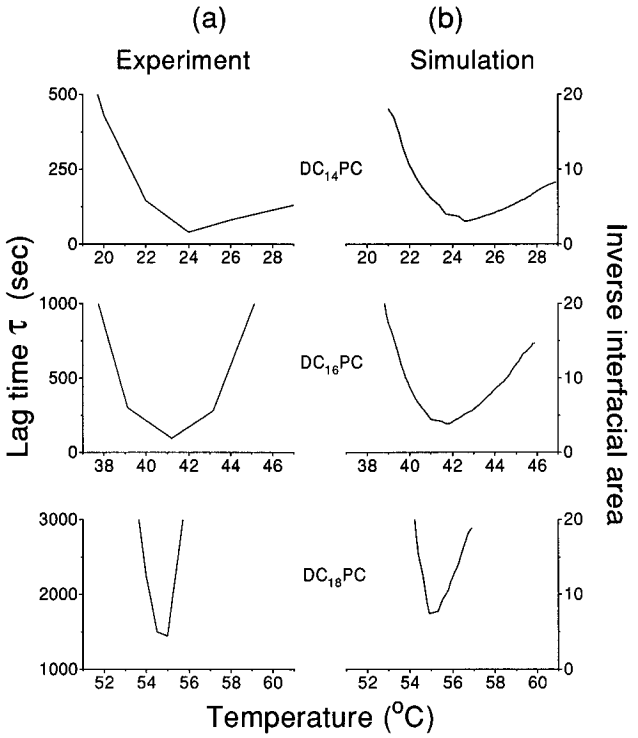

FIGURE 2: Correlation between the lag time, τ, and the microheterogeneity of the lipid bilayer. (a) Magnification of the lag-time curves shown in Figure 1b for the PLA_2-catalyzed hydrolysis of unilamellar vesicles in the temperature regions of the gel-to-fluid phase transition for $DC_{14}PC$, $DC_{16}PC$, and $DC_{18}PC$ lipid bilayers. (b) Monte Carlo computer simulation of the temperature dependence of the inverse interfacial area of lipid bilayers composed of DC_{14}-PC, $DC_{16}PC$, and $DC_{18}PC$ in their respective gel-to-fluid phase transition regions. The heterogeneous bilayer structure is divided into the bulk area, the domain area, and the interfacial area defined by the interfaces between the bulk and the domains.

T_m (a similar local maximum is resolvable for $DC_{14}PC$ when the reaction conditions are altered such that τ becomes longer at all temperatures). Figure 1b indicates that the local maximum in $\tau(T)$ is sharper and closer to T_m the longer the lipid chain is. $\tau(T)$ eventually increases at reaction temperatures further above T_m for all three series of data possibly due to an augmented fraction of denatured PLA_2 at high temperatures. On the basis of data covering a less extended temperature range, it has previously been stated that $\tau(T)$ is of parabolic-like form with a minimum at T_m (Apitz-Castro et al., 1982; Jain et al., 1989; Op den Kamp et al., 1974; Kensil & Dennis, 1979; Goormaghtigh et al., 1981; Lichtenberg et al., 1986) reflecting the time required to accummulate a so-called critical mole fraction of products in the lipid suspension (Apitz-Castro et al., 1982; Jain et al., 1989; Burack et al., 1993). However, the more extended $\tau(T)$ data in Figure 1b demonstrate a highly asymmetric, lipid chain length-dependent behavior of $\tau(T)$.

It has been proposed that so-called lipid-packing defects in the bilayer may promote high PLA_2 activity (Apitz-Castro et al., 1982; Op den Kamp et al., 1974; Kensil & Dennis, 1979; Goormaghtigh et al., 1981; Lichtenberg et al., 1986; Gabriel et al., 1987; Gheriani-Gruszka et al., 1988; Lehtonen et al., 1995). As suggested by previous work (Mouritsen & Jørgensen, 1992), we shall relate such packing defects to the structural microheterogeneity of the unhydrolyzed lipid bilayer. The microscopic structure and dynamics of the unhydrolyzed lipid bilayer near the phase transition can be obtained from computer simulation calculations on microscopic molecular interaction models (Mouritsen & Jørgensen, 1994). It is known from such simulation studies that the experimental observation of the characteristic lipid chain length dependence of macroscopic physical bilayer properties in the transition region, such as the thermal anomalies in the specific heat, the area compressibility, the bending rigidity, and the passive permeability (Bloom et al., 1991), reflects an underlying variation in thermal density fluctuations and hence in the microheterogeneity of the bilayer. The

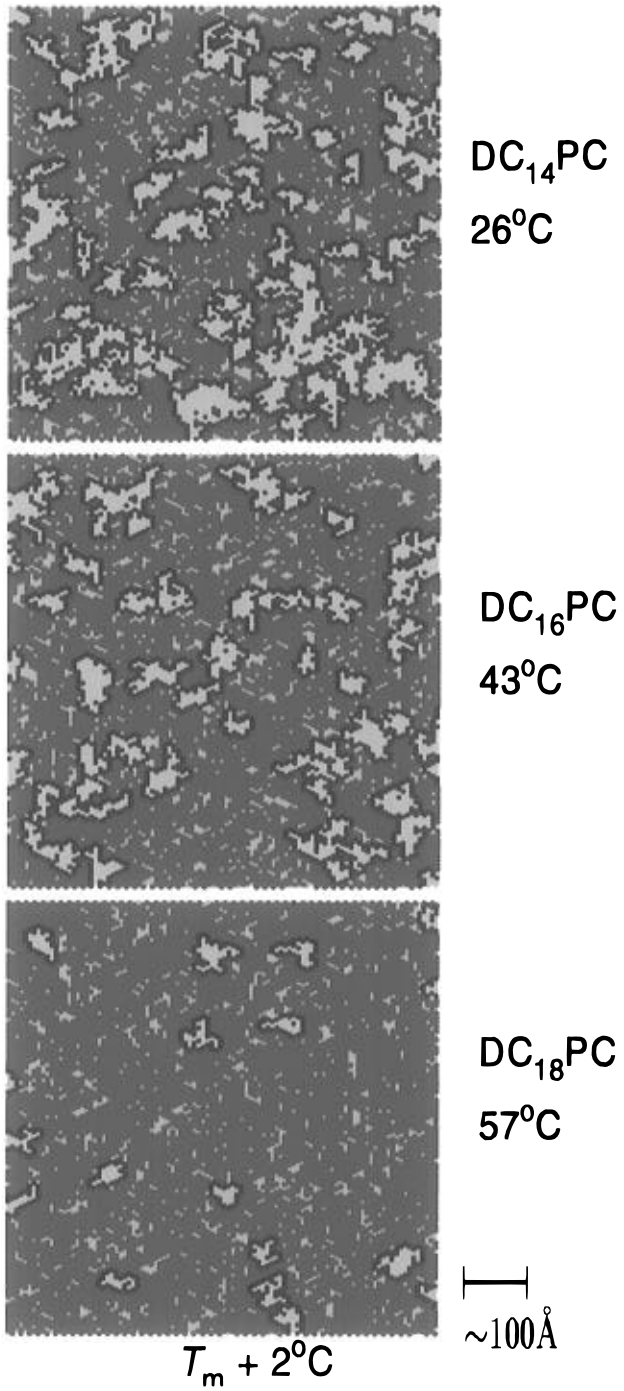

FIGURE 3: Dynamic lateral microheterogeneity of lipid bilayers as obtained by Monte Carlo computer simulations. The snapshots show typical schematic top-view bilayer configurations (550 Å × 550 Å) in the fluid phase 2 °C above the respective gel-to-fluid phase transition temperature, T_m, for the lipid bilayers composed of DC_{14}-PC, $DC_{16}PC$, and $DC_{18}PC$. Gel and fluid regions of the bilayer are denoted by yellow and red areas. The interfacial regions between the dynamic coexisting gel and fluid regions are highlighted in green.

closer to the phase transition and the shorter the lipid chain length, the stronger the microheterogeneity. This heterogeneity, which is of a dynamic nature, can be described in terms of the formation of lipid domains characterized by a length scale in the nanometer range as illustrated in Figure 3. The degree of heterogeneity can readily be quantified as the amount of bilayer area occupied by the interfaces between gel and fluid lipid domains. Figure 2b shows the calculated temperature and lipid chain length dependence of the inverse interfacial area as obtained from Monte Carlo computer simulations. By comparing the experimental data for $\tau(T)$ in Figure 2a with the simulated data for the inverse interfacial area in Figure 2b, we find a strong qualitative correlation between $\tau(T)$ and the degree of dynamic bilayer heterogeneity with respect to both temperature and lipid chain length variations. This correlation holds with respect to the width, the depth, and the asymmetry of the $\tau(T)$ function in the transition region. The comparison between the experimental and theoretical results in Figure 2 renders a correlation without addressing the specific molecular mechanisms behind the PLA_2 reaction time courses. A more quantitative interpretation of the experimental data in Figure 2a would require more information, e.g., about the actual molecular composition and morphology of the lipid substrate during and at the end of the lag phase including a clarification of the previously mentioned critical mole fraction of hydrolysis products (Burack & Biltonen, 1994; Bell et al., 1995).

The observation of a local maximum in $\tau(T)$ above T_m (cf. Figure 1b) has not been reported earlier. At present, the specific microscopic mechanisms involved in PLA_2-catalyzed hydrolysis of phospholipids are not fully known. Nevertheless, it is possible that the general behavior of $\tau(T)$ including this local maximum could be rationalized effectively in terms of the temperature and chain length dependence of a mesoscopic or macroscopic physical property of the bilayer. The bilayer bending rigidity, κ, is a candidate for such a property. Recent work shows that κ displays a maximum above T_m (Fernandez-Puente et al., 1994) and has been found to become anomalously low in the transition region due to a coupling between bilayer curvature and density fluctuations (Hønger et al., 1994).

The correlation found between PLA_2 activity and dynamic lipid bilayer microheterogeneity corroborates to the general hypothesis that the function of membrane-associated proteins can be modulated by collective physical properties of the lipid bilayer, specifically the dynamic bilayer heterogeneity in the nanometer range. The results from our combined experimental and theoretical approach furthermore suggest a rational way of learning about enzyme activity at lipid membranes in the presense of specific molecular compounds, e.g., membrane-active drugs and detergents (Mouritsen & Jørgensen, 1994; Gheriani-Gruszka et al., 1988; van Osdol et al., 1992) that are known to change the microstructure of lipid bilayer substrates.

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