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Increase in phospholipase A_2 activity towards lipopolymer-containing liposomes

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Abstract

Phospholipase A_2 (PLA_2)-catalyzed hydrolysis of dipalmitoylphosphatidylcholine (DPPC) liposomes incorporated with submicellar concentrations of polyethyleneoxide covalently attached to dipalmitoylphosphatidylethanolamine (DPPE-PEG_{2000}) has been studied in the gel-to-fluid transition region of the host DPPC lipid bilayer matrix. By means of fluorescence and light-scattering measurements, the characteristic PLA_2 lag time has been determined as a function of lipopolymer concentration and temperature. The degree of lipid hydrolysis was followed using radioactive labeled lipids. Differential scanning calorimetry has been applied to characterize the thermodynamic phase behavior of the lipopolymer-containing liposomes. A remarkable lipopolymer concentration-dependent decrease in the lag time was observed over broad temperature ranges. The radioactive measurements demonstrate an increase in catalytic activity for increasing amounts of lipopolymers in the bilayer. Hence, the lipopolymers act as a promoter of PLA_2 lipid hydrolysis resulting in a degradation of the bilayer structure and a concomitant destabilization of the liposomes. This behavior is in contrast to the generally observed protective and stabilization effect in biological fluids exerted by lipopolymers in polymer-grafted liposomes. It is proposed that the enhanced activity of the small water soluble and interfacially active enzyme may involve a non-uniform distribution of the lipopolymers in the lipid matrix due to a coupling between local lipid bilayer curvature and composition of the non-bilayer-preferring lipopolymers.

Keywords: Liposome; Phospholipase A_2; Enzyme-lipid interaction; Lipopolymer; Drug carrier; Lipid bilayer heterogeneity; Calorimetry; Fluorescence

1. Introduction

Liposomes are potential candidates as microcarrier systems for hydrophobic and hydrophilic drugs [1]. However, the utility of liposomes for delivering therapeutic agents has been severely limited due to a rapid removal and low circulation half-life, $t_{1/2}$, on the order of minutes in the blood stream [2,3]. An improvement in the liposome survival time in the blood stream to give $t_{1/2}$ values on the order of hours

Abbreviations: DPPC, dipalmitoylphosphatidylcholine; PLA_2, low molecular weight phospholipase A_2; DPPE-PEG_{2000}, polyethyleneoxide-dipalmitoylphosphatidylethanolamine with 45 monomers; PA, palmitic acid; lysoPC, L-α-lysopalmitoylphosphatidylcholine; ^{14}C-DPPC, 1-palmitoyl-2-[1-^{14}C]palmitylphosphatidylcholine; DSC, differential scanning calorimetry

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can be achieved by the use of small liposomes composed of high-melting phospholipid bilayers incorporated with high amounts of cholesterol [4], e.g. lipid bilayers in the so-called liquid-ordered phase characterized by tightly packed and ordered lipid acyl chains in a liquid-like bilayer matrix [5]. Both the macroscopic phase behavior of liposomes as well as the microscopic lipid bilayer organization are of importance for the in vitro behavior and the in vivo fate of liposomes, e.g. the long-term stability and interaction of liposomes with biological components as well as the distribution profile in different tissues. The prolongation of the circulation time of the liposomes in the blood stream can be understood on basis of an intimate relationship between the physicochemical, mechanical, and functional properties of lipid bilayers which are governed by the microstructural behavior of the composite lipid material [3,6,7].

A significant increase in the circulation time in the blood stream yielding $t_{1/2}$ values on the order of several hours can be obtained using surface-modified liposomes incorporated with glycolipid gangliosides, GM_1 [8]. Moreover, incorporation of synthetic polyethyleneoxide (PEG) lipopolymers into phospholipid liposomes with mean diameters less than 100 nm results in a further increase in the circulation time and an improvement in the in vivo behavior [9]. Surface-modified polyethyleneoxide liposomes better known as sterically stabilized liposomes have come into widespread use not only as particulate drug-delivery systems with improved therapeutic profiles [10,11], but also as model systems to study surface properties and phase behavior of polymer-grafted liposomes [12–17].

Despite intensive research activities in the field of sterically stabilized liposomes, the detailed mechanisms involved in the increased circulation time in the blood stream of PEG-grafted liposomes are still not fully understood [18]. The stabilization and protective role induced by the lipopolymers is generally considered to be the result of a steric barrier induced by the flexible PEG chains [10,18]. The steric barrier is believed to reduce the interactions between the polymer-grafted bilayer surface and liposome-degrading biological components. Hence, the repulsive interaction of the PEG polymer chains form a steric hindrance that prevents rapid recognition and removal of the liposomes by phacocytic cells of the reticoloendothelial system, RES [10,18]. It has been shown that liposomes incorporated with lipopolymers bind less plasma proteins and that the circulation time in the blood stream become shorter, when more proteins are adsorbed to the surface of the liposomes [9,19]. However, only a few studies have dealt with the interaction of well-defined liposome systems with membrane active and destabilization enzymes, such as phospholipases.

To gain further insight into the molecular origin and mechanisms underlying the prolongation of the circulation time in the blood stream and the extravascular degradation of polymer-grafted liposomes, we have undertaken a systematic investigation of the activity of PLA_2-catalyzed phospholipid hydrolysis of DPPC liposomes incorporated with DPPE-PEG_{2000} lipopolymers. PLA_2 belongs to a group of structurally related membrane active enzymes which are ubiquitously present in extra- and intracellular compartments of human tissues and also constitute the major component of several snake toxins [20,21]. The similarity of the amino acid sequences between mammalian and snake venom PLA_2 suggest common modes of lipid–membrane interactions and molecular mechanisms of the enzymatic phospholipid-catalyzed hydrolysis at the lipid–membrane interface [20,22]. PLA_2-catalysis involves adsorption of the enzyme to the lipid–membrane interface followed by hydrolysis of the phospholipids at the catalytic site of the enzyme. It is well established that the catalytic cleavage of phospholipids and the overall activity of PLA_2 depends strongly on the physical state and microstructure of the lipid bilayer substrate. Especially, it has been shown that the activity of PLA_2 is significantly enhanced in the phospholipid main-transition region where the activity correlates strongly with the formation of a heterogeneous lateral bilayer structure composed of coexisting dynamic lipid domains, which in turn may increase PLA_2-catalyzed phospholipid cleavage [23–25]. In addition, both the lateral surface stress and the incorporation of non-bilayer forming lipids into the bilayer matrix can give rise to an increase in the catalytic activity of PLA_2 [26,27].

Important drug-delivery aspects are related to an improved understanding of the relevant barrier prop-

erties of sterically stabilized liposomes that are involved in the in vivo destabilization and interaction of biological components with polymer-grafted liposomes. Although intensive research studies have been undertaken in the field of sterically stabilized liposomes a detailed understanding of the mechanisms that determine the in vivo destabilization and clearance is still lacking.

In this study, we have investigated the activity of PLA_2 towards DPPC liposomes incorporated with submicellar concentrations of DPPE-PEG_{2000} lipopolymers. The temperature dependence of the characteristic lag time, denoting the time required before a significant increase in the enzymatic activity takes place, has been monitored as a function of lipopolymer concentration of unilamellar extruded liposomes in the temperature region of the main phase transition of the host DPPC lipid bilayer matrix. In addition, the degree of PLA_2 catalyzed phospholipid hydrolysis has been followed after incorporation of radioactive ^{14}C-DPPC lipids into the lipid bilayer matrix.

2. Materials and methods

2.1. Materials

Polyethyleneoxide DPPE-PEG_{2000} (MW of PEG = 2053 g/mol, 45 monomers) lipopolymer and saturated DPPC phospholipid were purchased as powder from Avanti Polar Lipids (Birmingham, AL, USA). Palmitic acid (PA) and L-α-lysopalmitoylphosphatidylcholine (lysoPC) were purchased from Sigma (St. Louis, MO, USA). Radioactive-labeled DPPC (1-palmitoyl-2-[1-^{14}C]palmityl-PC, 2.18 GBq/mmol) was from Amersham (Little Chalfont, UK). Thin-layer chromatography plates (250-μm layer of Silica Gel 60) were from Merck (Damstadt, Germany) and Ecoscient scintillation fluid was obtained from National Diagnostics (Atlanta, GA, USA).

2.2. Preparation of unilamellar liposomes

To form multilamellar liposomes, weighed amounts of phospholipids and lipopolymers were dissolved and mixed in chloroform. The organic solvent was subsequently removed using a gentle stream of clean air leaving a thin lipid film, which was dried overnight at low pressure to remove any trace impurities of the organic solvent. The multilamellar liposomes were made by dispersion of the dried lipid in buffer solution. The lipid suspension was kept for at least 1 h at 51°C to assure complete hydration. During this period, the lipid suspension was vortexed several times. Unilamellar liposomes of narrow size distribution were made from the multilamellar liposomes by extrusion of the multilamellar samples ten times through two stacked 100 nm pore size polycarbonate filters [28].

2.3. Differential scanning calorimetry and dynamic light scattering

Differential scanning calorimetry of 0.15 mM unilamellar liposomes was performed using a MicroCal MC-2 (Northhampton, MA) scanning calorimeter in the upscan mode at a scan-rate of 10°C/h. An appropriate baseline has been subtracted from the calorimetric curves, whereas no correction was made for the fast response time of the power-compensating calorimeter. Size measurements of the unilamellar liposomes were performed using a Zetasizer 4 (Malvern, UK). The dynamic light scattering data were collected at an angle of 90° using a helium laser as light source. The liposomes were stored at 4°C between the size measurements. The apparatus was calibrated by measuring mono-dispersed polystyrene latex-spheres of known size (220 nm).

2.4. PLA_2 (*Naja naja naja*) radioactive phospholipid hydrolysis

PLA_2 (*N. naja naja*) for the radioactive phospholipid hydrolysis measurements was purchased from Sigma Chemical Co. and used without further purification. Assay conditions for the PLA_2 radioactive phospholipid hydrolysis included 2 mM unilamellar liposomes, 0.49 μM PLA_2, 10 mM $CaCl_2$, 135 mM KCl, 9 mM HEPES (pH 7.5), and 0.9 μM EDTA. The catalytic reaction was initiated by adding 5 μl of a 49 μM PLA_2 stock solution to 0.495 ml liposome suspension thermostated at 41°C. The concentration

of radioactive-labeled ^{14}C-DPPC lipid was 0.06 mol%. The enzymatic reaction was stopped after 20 min by the addition of 0.5 ml chloroform/methanol/acetic acid (2:4:1 v/v/v). A 0.5 ml amount of chloroform was added to extract the lipids to the organic phase. The mixture was briefly vortexed and centrifuged to separate the organic and aqueous phase. The organic layer was subsequently removed and dried using a gentle stream of clean air. The lipid extraction procedure was repeated three times. A 200 μl amount of chloroform/methanol (1:1) was added to dissolve the lipid residue. A 30 μl amount of the dissolved and hydrolyzed lipid sample was loaded onto a thin-layer chromatography plate (one lane of 2 cm width), and the lipids were separated over 16.5 cm by elution with chloroform/methanol/acetic acid/water (25:15:4:2, v/v/v/v). The lipids were exposed to I_2 vapor, and the separated lipid zones were scraped directly into scintillation vials into which 3 ml of scintillation fluid was added. The ^{14}C radioactivity was determined using a Minaxi Tri-carb 4000 scintillator, United Technologies, Packard.

2.5. PLA_2 (*Agkistrodon piscivoros piscivoros*) lag time measurements

Purified PLA_2 (*A. piscivoros piscivoros*) for the lag time measurements was a gift from Professor R.L. Biltonen, University of Virginia. PLA_2 has been isolated and purified according to the procedure of Maraganore et al. [29]. Assay conditions for the PLA_2 lag time measurements were 0.15 mM unilamellar liposomes, 150 nM PLA_2, 150 mM KCl, 10 mM HEPES (pH 7.5), 1 mM NaN_3, 30 μM $CaCl_2$, and 10 μM EDTA. The catalytic reaction was initiated by adding 8.3 μl of a 45 μM PLA_2 stock solution to 2.5 ml of the thermostated lipid suspension equilibrated for at least 20 min prior to addition of the enzyme. The PLA_2 reaction profile and lag time denoting the time required before the onset in rapid enzymatic activity takes place, was defined on basis of a sudden increase in the intrinsic fluorescence from PLA_2 emitted at 340 nm after excitation at 285 nm and a decrease in 90° static light scattering from the lipid suspension. The fluorescence and scattering data were simultaneously measured using a SLM DMX-1100 spectrofluorometer.

3. Results and discussion

3.1. Heat capacity and liposome size

Fig. 1 shows differential scanning calorimetry results obtained at a scan rate of 20°C/h for pure one-component DPPC unilamellar liposomes and DPPC liposomes incorporated with DPPE-PEG_{2000} lipopolymers in two different concentrations. The main transition temperature, T_m, for pure DPPC unilamellar liposomes takes place at 41.15°C as determined from the peak position of the heat capacity, C_p. The minor peak on the upper temperature side of the main peak reflects a trace impurity of multilamellar liposomes in the suspension. The C_p curves for the unilamellar DPPC liposomes incorporated with 2.5 and 5 mol% DPPE-PEG_{2000} lipopolymer are only weakly distorted as compared to the C_p curve for pure 100 nm unilamellar DPPC liposomes. The main transition temperature, T_m, defined as the peak maximum of the heat capacity curves in Fig. 1 is found to be rather insensitive to composition of

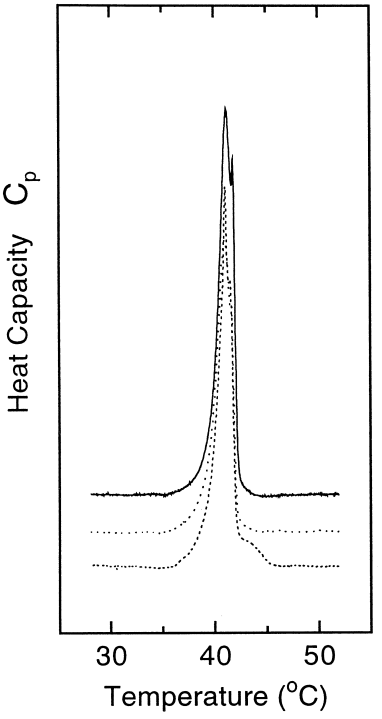

Fig. 1. Differential scanning heat–capacity curves obtained at a scan rate of 20°C/h for DPPC unilamellar liposomes containing 0 mol% (solid line), 2.5 mol% (dotted line), and 5 mol% (dashed line) DPPE-PEG_{2000} lipopolymers.

the lipopolymer-containing liposomes. This behavior is in accordance with thermodynamic data for 50 nm extruded liposomes [17] and furthermore is to be expected when the lipopolymer concentration is below the threshold concentration, n_{tr}, for the lipid bilayer to lipid micellar transition. Both theoretical and experimental investigations of mixed liposome systems have estimated the micellar threshold value to be around 10 mol% [13,14,17]. A change in the configuration of the polymer chains from a so-called mushroom to a brush structure is expected to take place when the DPPE-PEG_{2000} concentration is increased from 2.5 to 5 mol% [15,16].

Dynamic light scattering results of the time development of the average particle size of extruded liposomes is shown in Fig. 2 for different concentrations of DPPE-PEG_{2000} lipopolymer in the lipid bilayer. A significant increase in the characteristic size of the pure DPPC liposomes takes place within the first few days after extrusion possibly due to fusion and a concomitant release of the curvature built in stress of the extruded liposomes. This behavior is opposite to DPPC liposomes incorporated with increasing concentrations of DPPE-PEG_{2000} lipopolymers. The presence of the lipopolymers in the bilayer leads to a short-term as well as a long-term stabilization of the size of the liposomes. The polymer-grafted liposome surface forms a steric barrier that prevent liposome–liposome contact which is a prerequisite for the formation of larger size liposomes due to aggregation and/or fusion of the liposomes [30]. Moreover, it is to be expected that the micellar-preferring lipopolymers decrease the curvature stress and thereby stabilize a smaller size of the extruded liposomes.

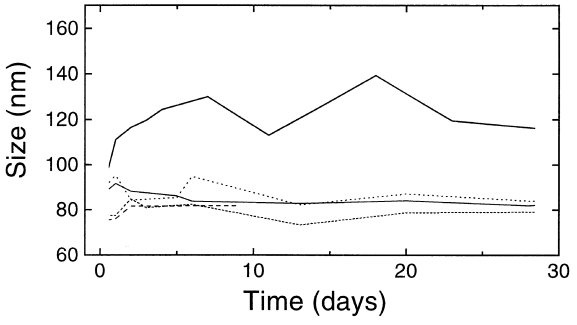

Fig. 2. Time development of the average particle size of DPPC phospholipid liposomes containing 0 mol% (heavy solid line), 0.5 mol% (short dashed line), 2.5 mol% (dotted line), 5 mol% (dashed line), and 7 mol% (thin solid line) DPPE-PEG_{2000} determined by dynamic light scattering measurements.

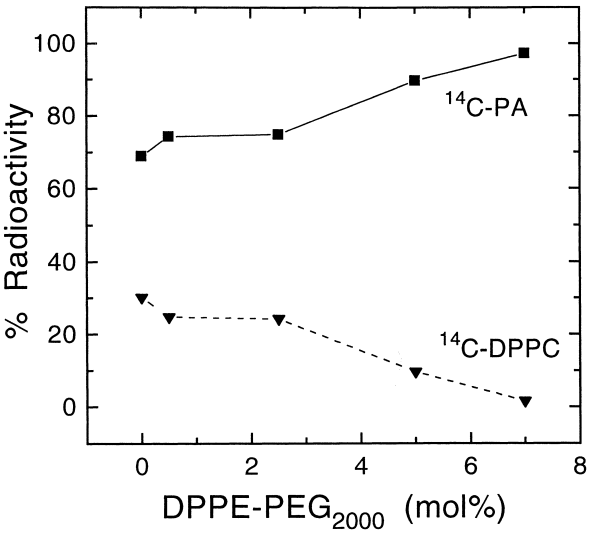

Fig. 3. PLA_2 (*N. naja naja*) hydrolysis of DPPE-PEG_{2000} lipopolymer-containing liposomes at 41°C as determined by percent of radioactive phospholipids (^{14}C-DPPC) and fatty acids (^{14}C-PA) 20 min after adding the enzyme to the liposome suspension.

3.2. PLA_2 (*N. naja naja*) lipid hydrolysis

Fig. 3 shows the degree of PLA_2 (*N. naja naja*) lipid hydrolysis of the liposome suspensions 20 min after addition of the enzyme as determined by radioactive lipid measurements. A remarkably increase in the degree of lipid hydrolysis takes place for increasing concentrations of DPPE-PEG_{2000} in the liposomes. When 7 mol% of DPPE-PEG_{2000} is incorporated into the lipid bilayer an almost complete PLA_2-catalyzed lipid hydrolysis of the liposomes takes place. We shall below return to a more detailed discussion of the influence of DPPE-PEG_{2000} lipopolymers on the enhancement of PLA_2 activity which might be closely related to the structural organization of the lipopolymers in the bilayer matrix.

3.3. PLA_2 (*A. piscivorus piscivorus*) lag time results

Typical PLA_2 (*A. piscivorus piscivorus*) hydrolysis time course profiles obtained at 39°C are shown in Fig. 4 for DPPC unilamellar liposomes incorporated with 0, 2.5, and 5 mol% DPPE-PEG_{2000} lipopolymers. The lag time, τ, which is defined on basis of

a sudden increase in the rate of hydrolysis, is determined by a rapid increase in the intrinsic PLA_2 tryptophan fluorescence intensity [23,27,31] and a concomitant decrease in 90° static light scattering

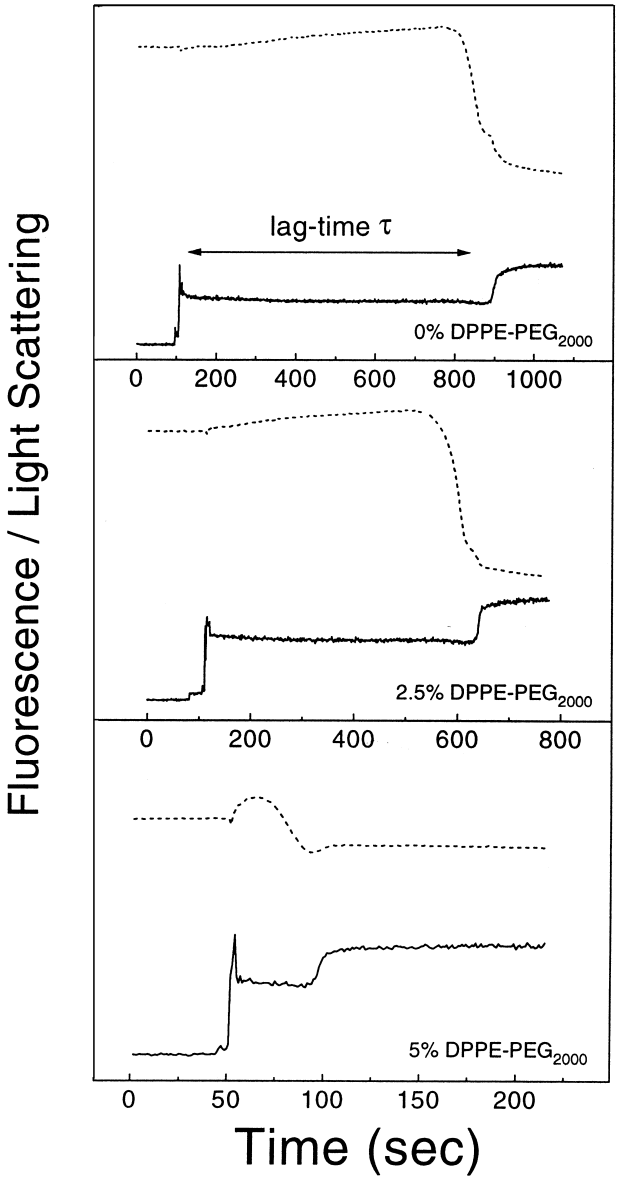

Fig. 4. Characteristic reaction time profiles at 39°C for PLA_2 (*A. piscivorus piscivorus*) hydrolysis of unilamellar DPPC liposomes incorporated with 0, 2.5, and 5 mol% DPPE-PEG_{2000} lipopolymers. The PLA_2 hydrolysis reaction is monitored by intrinsic fluorescence (solid line) from the enzyme and 90° static light scattering (dashed line) from the suspension. After adding PLA_2 to the equilibrated liposome suspension a characteristic lag time, τ, follows before a sudden increase in the catalytic activity of the enzyme takes place [23,24].

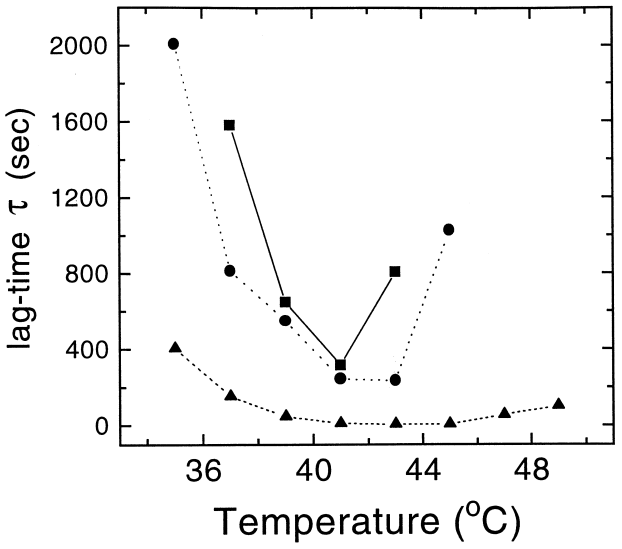

Fig. 5. PLA_2 (*A. piscivorus piscivorus*) lag time, τ, as a function of temperature for the hydrolysis of DPPC liposomes incorporated with 0 mol% (solid line), 2.5 mol% (dotted line), and 5 mol% (dashed line) DPPE-PEG_{2000} lipopolymers.

caused by a change in the morphology of the lipid system [24,25]. In Fig. 5, the lag time results are shown as a function of temperature in the gel-to-fluid transition of unilamellar liposomes composed of pure DPPC, and for liposomes incorporated with 2.5 and 5 mol% DPPE-PEG_{2000} lipopolymer. Each of the three lag time curves shown in Fig. 5 displays a minimum in the temperature region of the gel-to-fluid phase-transition temperature, T_m, as determined by the C_p curves in Fig. 1. These minima become deeper and broader for increasing concentrations of DPPE-PEG_{2000} lipopolymer in the liposomes. It is well-known that the lag time, τ, for pure phospholipid liposomes obtains a minimum at the gel-to-fluid transition, T_m, [24,32,33] possibly reflecting the time required to accumulate a certain amount of hydrolysis products in the lipid system [23,33]. The results shown in Fig. 5 clearly reveal that a remarkably lipopolymer concentration-dependent lowering of τ takes place over broad temperature ranges in the transition region. The significant effect of DPPE-PEG_{2000} lipopolymers on the PLA_2 lag time is further emphasized by the data in Fig. 6, which shows the influence of increasing lipopolymer concentrations at a fixed temperature, $T = 41$°C, corresponding to the minimum value of τ.

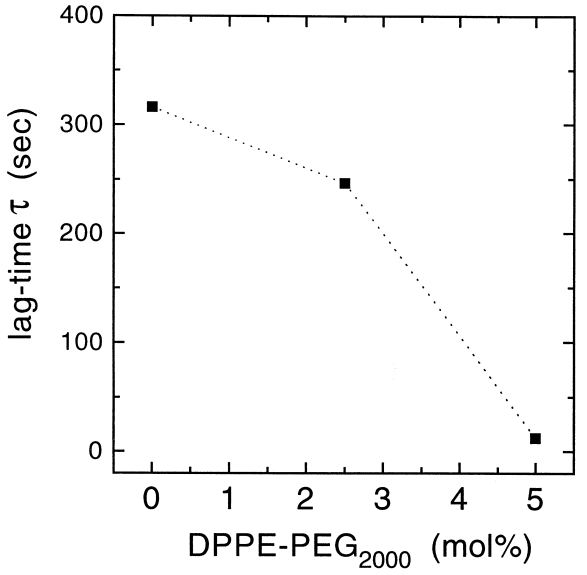

Fig. 6. PLA_2 (*A. piscivorus piscivorus*) lag time, τ, as a function of DPPE-PEG_{2000} lipopolymer concentration obtained at 41°C.

3.4. Modulation of PLA_2 activity towards polymer-grafted liposomes.

To provide a unified interpretation of the influence of submicellar DPPE-PEG_{2000} lipopolymer concentrations on the activity of PLA_2 in the temperature region of the gel-to-fluid phase transition (cf. Fig. 3 and Fig. 5), we will make a simple assumption that relates the lateral organization of the unhydrolyzed lipid bilayer to the increase in PLA_2 enzymatic activity. The existence of a heterogenous lipid bilayer structure composed of lipid domains and interfacial regions characterized by lipid packing defects have been suggested to promote PLA_2-catalyzed phospholipid hydrolysis [26,32,34]. The incorporation of submicellar lipopolymer concentrations into lipid bilayers is expected to give rise to the formation of local phase-separated regions which are characterized by a length scale in the nanometer range due to an intimate relationship between composition and curvature of the lipid matrix [35–37]. When low concentrations of DPPE-PEG_{2000} lipopolymers are incorporated into DPPC lipid bilayers the two components are expected to be more or less uniformly distributed in the lipid matrix due to the entropy of mixing. As the lipopolymer concentration increases, a curvature frustration is built up, where the two components will compete for lipid bilayer regions of varying local curvature [38]. Formation of lipid bilayer regions of high curvature might therefore function as aggregation sites for the non-bilayer-preferring DPPE-PEG_{2000} lipopolymers and furthermore give rise to curvature induced phase-separation phenomena [35,36]. This leads to a heterogeneous bilayer structure composed of coexisting lipid domains and interfacial regions with phospholipids which may exhibit an increased susceptibility to PLA_2-catalyzed hydrolysis.

The formation of a heterogeneous lateral structure and a network of interfacial regions with the lipopolymers segregated into regions of high curvature of the undulating lipid bilayer might be an important regulator of PLA_2 activity. The results suggest that submicellar lipopolymer concentrations act as a promoter of PLA_2 lipid hydrolysis resulting in a destabilization of the bilayer structure by the hydrolysis products and a concomitant degradation of the liposomes. At this point in our investigation, however, it is not clear if the non-bilayer-perturbing effect of DPPE-PEG_{2000} lowers the critical mole fraction of hydrolysis of the lipid suspension [31] or simply accelerates the process as suggested above. The observed enhancement in catalytic activity (cf. Fig. 3 and Fig. 5) is opposite to the generally observed protective and stabilizing effect exerted by lipopolymers in the blood stream, e.g., the lipopolymers establish a steric hindrance which leads to a decrease in the adsorption and interaction of plasma degrading proteins with the liposome surface [9,19]. An important factor involved in destabilization of liposomes in the blood stream is the physical adsorption of proteins and enzymes to the liposome surface, e.g. transfer of lipids by lipoproteins and the activity of lipolytic enzymes. However, the role of especially phospholipases in the blood stream is considered to be minimal [39] as suggested by the results reported above. It is proposed that the enhanced in vitro catalytic activity of the small water soluble and interfacial active PLA_2 enzyme observed in this work reflects an intimate relationship between local lipid bilayer curvature and composition of the non-bilayer-preferring lipopolymers. The formation of a heterogeneous lateral bilayer structure composed of lipid domains and interfacial regions with lipid packing defects may hence facilitate PLA_2 lipid hydolysis by making the interfacial phospholipids more acces-

sible to the catalytic site of the enzyme. Other factors which might contribute to the increased PLA_2 hydrolysis towards PEG-containing liposomes compared to pure DPPC liposomes involve differences in liposome size and lipid packing properties caused by the incorporation of PEG-$DPPE_{2000}$ lipids into the bilayer matrix. It has been shown earlier that the lag time of PLA_2-catalyzed phospholipid hydrolysis is directly proportional to the size of the liposomes [40]. In addition, the hydrophilicity of the lipopolymer network may cause a change in the affinity of the small water soluble PLA_2 enzyme to the polymer-grafted lipid bilayer surface, e.g. an increase in the binding and contact time of the enzyme to the liposomes.

The results presented above suggest that the enhanced catalytic hydrolysis of phospholipids by PLA_2 in polymer grafted liposomes is unlikely to be involved in the prolonged circulation time in the blood stream of sterically stabilized liposomes. In contrast, an increase in the degradation of polymer grafted liposomes in extravascular pathological tissue is to be expected due to a combined effect of an elevated and high concentration of active PLA_2 [41,42], and an increased catalytic activity towards lipopolymer containing liposomes as suggested by our results.

4. Conclusion

In this study, we have investigated the influence of lipopolymer-containing liposomes on the activity of PLA_2. The results demonstrate an increase in the susceptibility of the phospholipids to PLA_2 catalytic cleavage for increasing amounts of submicellar concentrations of lipopolymer in the lipid bilayer. It is suggested that the enhanced enzymatic activity of PLA_2 is caused by a change in the microscopic lateral organization of the lipid bilayer structure. Insight into microscopic and macroscopic bilayer properties that control the enzymatic phospholipid hydrolysis and the overall destabilization of liposomes plays a crucial role for the potential use of liposomes as particulate drug-delivery systems [7]. In a broader context, the results presented above may help clarifying the relationship between membrane associated functions and structural properties of lipid bilayers that are of significant basic and biotechnological interest. A fundamental understanding of the influence of composition on functional biomaterial properties of composite lipid systems are of relevance for a rational modification and optimization of liposomes as drug-carrier systems. The results presented above might advantageously be used to design and optimize the in vivo degradation of drug loaded liposomes at certain sites, e.g. in extravascular inflammatory tissue due an enhanced local concentration of active PLA_2 and an accumulation of polymer-grafted liposomes in such tissue. The results may furthermore have implications for a deeper understanding of the regulation of enzymatic functions via modification of the physical properties of the lipid bilayer component of biological membranes [25,43].

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