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Short communication

Interaction of a lipid-membrane destabilizing enzyme with PEG-liposomes☆

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Abstract

Polymer grafted PEG-liposomes have come into use as drug-delivery systems with improved therapeutic profiles. However, very little is known about the morphological instability of PEG-liposomes due to enzymatic degradation. To gain further insight into the effect of PEG lipopolymer-concentration on the catalytic activity of a liposome-degrading enzyme, phospholipase A_2 (PLA_2)-catalyzed phospholipid hydrolysis of PEG-liposomes has been investigated. The temperature dependence of the PLA_2 lag-time, denoting the time required before a sudden increase in enzymatic activity takes place, has been determined for submicellar amounts of dipalmitoylphosphatidylethanolaminyl-poly-(ethylene glycol) (DPPE-PEG_{2000}) incorporated into unilamellar dipalmitoylphosphatidylcholine (DPPC)-liposomes. The measurements demonstrate a significant reduction in the lag-time over broad temperature ranges. The results suggest that a close relationship exists between PLA_2 catalyzed lipid hydrolysis and lipid-membrane composition, which moreover is of major importance for the overall morphological stability and the release of encapsulated material from the polymer-grafted PEG-liposomes.

Keywords: PEG-liposome; Drug-delivery; Phospholipase A_2; Liposome-degradation; Enzyme-membrane interaction

The utility of liposomes for delivering therapeutic agents has been severely limited due to a rapid removal and low circulation half-life in the blood-stream (Senior, 1987). A significant improvement in the liposome survival-time in the blood-stream can be achieved by the use of surface-modified

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PEG-liposomes containing synthetic polyethyleneoxide lipopolymers (Lasic and Martin, 1995). Despite intensive research activities in the field of sterically stabilized PEG-liposomes the detailed mechanisms involved in the intra- and extravascular degradation are still not fully understood. The stabilization and protective role induced by the lipopolymers is generally considered to be the result of a steric barrier induced by the flexible polymer chains (Baekmark et al., 1993; Blume and Cevc, 1993; Hristova et al., 1995; Kenworthy et al., 1995).

Important drug-delivery aspects are related to an improved understanding of the relevant barrier properties of sterically stabilized PEG-liposomes that are involved in the interaction and destabilization by biological components (Lasic and Martin, 1995). To gain further insight into the mechanisms underlying the intra- and extravascular degradation of polymer-grafted PEG-liposomes, phospholipase A_2 (PLA_2) catalyzed hydrolysis of dipalmitoylphosphatidylcholine (DPPC) liposomes incorporated with a small amount of dipalmitoylphosphatidylethanolaminyl-poly-(ethylene glycol) (DPPE-PEG_{2000}) has been investigated. PLA_2 belongs to a group of lipid-membrane active enzymes, which are present in variable concentrations in different compartments of human tissue (Hansen et al., 1995). 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-membrane substrate (Burrack et al., 1993; Hønger et al., 1996; Mouritsen and Jørgensen, 1997). PLA_2 catalysis involves adsorption of the enzyme to the lipid-membrane interface followed by phospholipid-hydrolysis at the catalytic site of the enzyme. The characteristic PLA_2 lag-time, denoting the time required before a significant increase in the enzymatic activity takes place, has been monitored in the temperature region of the main phase-transition when submicellar amount of DPPE-PEG_{2000} is incorporated into the host DPPC lipid-membrane matrix.

Multilamellar liposomes composed of DPPC and DPPE-PEG_{2000} (MW of PEG = 2053 g/mol, 45 monomers) were made by hydration of dried lipid films. The lipid suspension was kept for at least 1 h at 51°C to assure complete hydration. The lipids were from Avanti Polar Lipids (Birmingham, AL). 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.

Purified PLA_2 (*Agkistrodon piscivoros piscivoros)* for the lag-time measurements was a gift from Professor R.L. Biltonen, University of Virginia. 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, was defined on basis of a sudden decrease in 90° static light scattering and an increase in the intrinsic fluorescence from PLA_2 emitted at 340 nm after excitation at 285 nm. The fluorescence and light scattering data were simultaneously measured using a SLM DMX-1000 fluorometer.

Fig. 1 shows the temperature dependence of the PLA_2 lag-time in the temperature range of the main-transition for pure unilamellar DPPC liposomes and for PEG-liposomes incorporated with 5 mol% DPPE-PEG_{2000}. Both curves display a minimum in the temperature region of the gel-to-fluid main-transition region. It is well-established that the lag-time, τ, for pure phospholipid liposomes obtains a minimum at the gel-to-fluid main-transition, T_m, (Op den Kamp et al., 1974; Hønger et al., 1996) reflecting the time required to accumulate a certain amount of hydrolysis products in the lipid system (Burrack et al., 1993). The results shown in Fig. 1 clearly reveal that the incorporation of DPPE-PEG_{2000} into the liposomes leads to a remarkably lowering of the lag-time, τ, over broad temperature ranges (Vermehren et al., 1998).

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 (Op den Kamp et al., 1974; Hønger et al., 1997). It is likely that the incorporation of submicellar concentrations of DPPE-PEG_{2000} into the liposomes might give rise to the formation of local phase-separated regions due to relationship between composition and curvature of the lipid-membrane matrix (Brumm et al., 1996; Warriner et al., 1996). When low concentrations of DPPE-PEG_{2000} lipopolymers are incorporated into DPPC liposomes, the two lipid-membrane components are expected to be more or less uniformly distributed in the lipid matrix. As the lipopolymer-concentration increases a curvature frustration is created, where the two components will compete for lipid-membrane regions of varying local curvature (Leibler, 1986). The formation of lipid-membrane regions of high curvature may function as aggregation sites for the non-bilayer preferring DPPE-PEG_{2000} lipopolymers and furthermore give rise to curvature induced phase-separation phenomena (Brumm et al., 1996; Warriner et al., 1996).

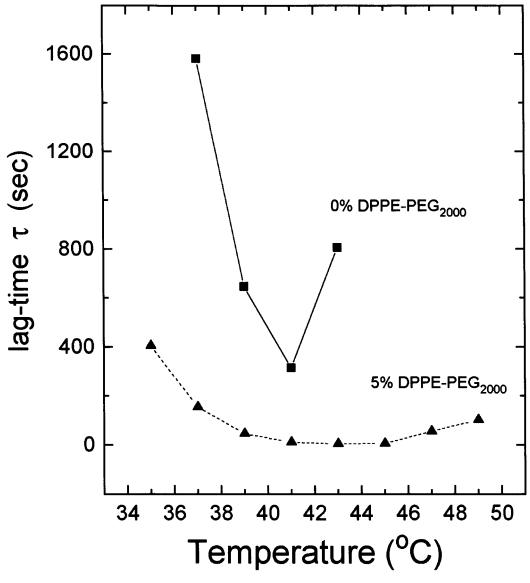

Fig. 1. Phospholipase A_2 (PLA_2) (*Agkistrodon piscivorus piscivorus*) lag-time, τ, as a function of temperature for pure unilamellar dipalmitoylphosphatidylcholine (DPPC) liposomes (solid line) and PEG-liposomes containing 5 mol% DPPE-PEG_{2000} (dotted line).

The obtained results suggest that the presence of submicellar amounts of the lipopolymer act as a promoter of PLA_2-catalyzed lipid hydrolysis resulting in a destabilization of the lipid-membrane structure by the fatty-acid and lyso-lipid hydrolysis products. However, it is not clear if the non-bilayer perturbing effect of DPPE-PEG_{2000} lowers the critical mole fraction of hydrolysis products (Burrack et al., 1993) or simply accelerates the formation of hydrolysis products.

An important factor involved in the destabilization of liposomes in the blood-stream is the physical adsorption of proteins and enzymes to the liposome surface (Chonn et al., 1992). However, the role of especially phospholipases in the blood-stream is considered to be minimal as suggested by the results reported above. Accordingly, the obtained in vitro results suggest that the enhanced catalytic activity of PLA_2 towards PEG-liposomes might be of importance for the degradation of polymer-grafted liposomes in extravascular pathological tissue due to a combined effect of an elevated and high concentration of active PLA_2 and an accumulation of polymer-grafted PEG-liposomes in such tissue (Lasic and Martin, 1995). The obtained results can advantageously be used to design and optimize the in vivo degradation of drug loaded PEG-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.

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