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Drug delivery by phospholipase A_2 degradable liposomes☆

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Abstract

The effect of poly(ethylene glycol)-phospholipid (PE-PEG) lipopolymers on phospholipase A_2 (PLA_2) hydrolysis of liposomes composed of stearoyl-oleoylphosphatidylcholine (SOPC) was investigated. The PLA_2 lag-time, which is inversely related to the enzymatic activity, was determined by fluorescence, and the zeta-potentials of the liposomes were measured as a function of PE-PEG lipopolymer concentration. A significant decrease in the lag-time, and hence an increase in enzymatic activity, was observed with increasing amounts of the negatively charged PE-PEG lipopolymers incorporated into the SOPC liposomes. The enhancement of the PLA_2 enzymatic activity might involve a stronger PLA_2 binding affinity towards the negatively charged and polymer covered PEG liposomes.

Keywords: Drug-delivery; PEG liposomes; Phospholipase A_2; Lipopolymer; Liposomes; Degradation

Drug-delivery systems based on liposomes incorporated with polyethylene glycol (PEG)-linked lipids are effective microcarrier system that can retain encapsulated drug, evade rapid clearance from the circulation, and passively target the interstitial tissue of solid tumors (Needham, 1999). However, the final challenges for effective liposomal drug delivery consists of controlling the destabilization, release and absorption of drug at the target site, e.g. in the interstitial tumor tissue. Different strategies for controlling and obtaining a burst drug release have been developed, including release triggered by the use of environmental-sensitive liposomes that respond to changes in pH (Conner et al., 1984) and release triggered by local intervention techniques such as hyperthermia (Yatvin et al., 1978; Needham et al., 2000).

Another strategy for controlled release and absorption takes advantages of endogenous phospholipases, e.g. phospholipase A_2 (PLA_2), which is found in elevated concentrations in various types of cancer (Abe et al., 1997). PLA_2 catalyzes the hydrolysis of the ester linkage of the *sn*-2 acyl chain of phospholipids, yielding free fatty acids and 1-acyl-lysophospholipids. The overall activity

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of PLA_2 depends strongly on the physical state and the microstructure of the liposome, which are determined by the lipid composition of the membrane (Burack et al., 1993; Hønger et al., 1996; Mouritsen and Jørgensen, 1998). It has been shown that it is possible to control and enhance the hydrolytic activity of PLA_2 by incorporation of submicellar concentrations of (PEG)-linked lipids into unilamellar dipalmitoylphosphatidylcholine (DPPC) liposomes (Vermehren et al., 1998; Jørgensen et al., 1999a,b). PLA_2-triggered release has an additional advantage compared with other triggered release strategies. When the liposomal membrane is destabilized by the action of PLA_2 and the drug is subsequently released, lysolipids and free fatty acids are generated at the same time in locally high concentrations in a one-to-one molar ratio. It is expected that the generated lysolipids and fatty acids act as permeability enhancers and therefore increase drug transport across the biological membrane.

In the present work, we have investigated how incorporation of small concentrations of PEG-linked lipids influences the PLA_2-catalyzed hydrolysis of liposomes composed of stearoyl-oleoylphosphatidylcholine (SOPC). Furthermore, we have measured the surface potential (zeta-potential) of the PEG-grafted SOPC liposomes.

Unilamellar fully hydrated liposomes with a narrow size distribution were made from various concentrations of SOPC and Polyethyleneoxide-distearoylphosphatidylethanolamine (DSPE-PEG_{750}) (Avanti Polar Lipids, Alabaster, AL) as described by Hope et al. (1985). Purified snake-venom PLA_2 (*Agikistrodon piscivorus piscivorus*) for the lag-time measurements was a gift from 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 ethylenediamine tetraacetic acid. The catalytic reaction was initiated by adding 8.9 μl of a 42 μM PLA_2 stock solution to 2.5 ml thermostated liposome suspension equilibrated for at least 20 min at 31°C prior to addition of PLA_2. Zeta-potentials were measured by laser Doppler electrophoresis using a ZetaMaster (Malvern Instruments Ltd. UK) in the same HEPES buffer (pH 7.5) as used in the PLA_2 lag-time measurements.

Fig. 1 shows the PLA_2 lag-time, τ, as a function of the concentration of DSPE-PEG_{750}. The lag-time, which is defined as the time elapsed after addition of the enzyme until the onset of rapid hydrolysis takes place, is determined by a sudden increase in the intrinsic PLA_2 tryptophan fluorescence intensity (Burack et al., 1993). The curve in Fig. 1 shows a decrease in τ, reflecting an enhanced PLA_2 hydrolytic activity when the DSPE-PEG is incorporated into the membrane. The decrease in the lag-time as a function of lipopolymer concentration is in agreement with earlier PLA_2 lag-time measurements on saturated DPPC liposomes incorporated with increasing lipopolymer concentrations (Vermehren et al., 1998; Jørgensen et al., 1999a,b)

Fig. 2 shows the zeta-potential as a function of increasing concentrations of DSPE-PEG_{750}. The decrease in the zeta-potential for the PEG-grafted SOPC liposomes correlates with the observed increase in PLA_2 activity reflected as a decrease in the lag-time shown in Fig. 1. It is possible that induction of a negatively charged lipid membrane surface facilitates the binding of PLA_2 and thereby increases the activity of the enzyme. In addition, it is likely that the large hydrophilic polymer headgroup influences the kinetics of the PLA_2 diffusion characteristic close to the lipid

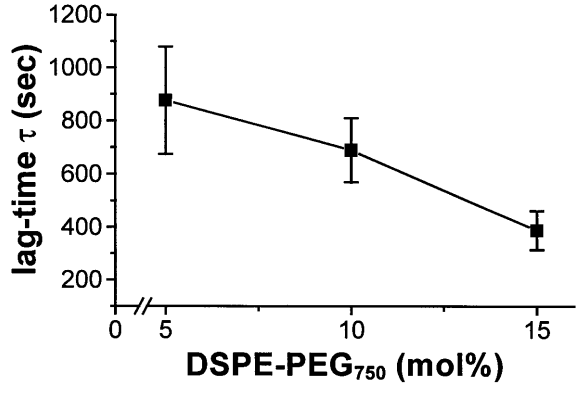

Fig. 1. Phospholipase A_2 (*A. piscivorus piscivorus*) lag-time, τ, of SOPC liposomes as function of DSPE-PEG_{750} lipopolymer concentration obtained at 31°C at pH 7.5.

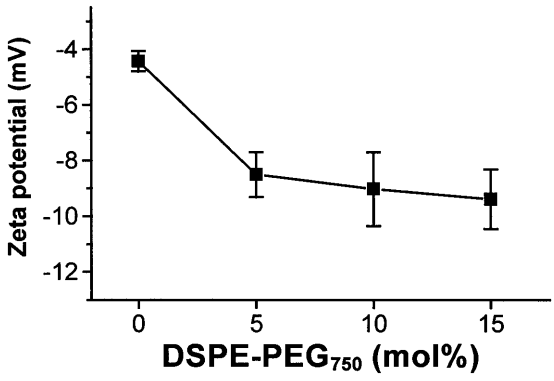

Fig. 2. Zeta-potential of SOPC liposomes as a function of DSPE-PEG_{750} lipopolymer concentration measured in HEPES buffer (pH 7.5).

membrane surface. It has previously been shown that the hydrolytic activity of PLA_2 depends strongly on the physical properties of the lipid membrane substrate (Burack et al., 1993; Burack and Biltonen, 1994; Hønger et al., 1996). Especially, the lateral organization of the lipid membrane is of importance (Hønger et al., 1996, 1997).

The obtained results show that it is possible to control and enhance the hydrolytic activity of PLA_2 towards the polymer-grafted PEG-SOPC liposomes. Furthermore, the advantage of the locally generated permeability enhancers, e.g. lysolipids and free fatty acids, on the transport of the released drug across the target membrane was investigated in a model assay composed of non-hydrolyzable etherlipid liposomes with encapsulated calcein in a self-quenching concentration. The assay measured the permeability enhancing effect of the PLA_2-catalyzed hydrolysis products towards a model membrane system (Davidsen et al., unpublished observations). It was found that the non-bilayer forming hydrolysis products induce a rapid increase in the trans-membrane permeability.

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