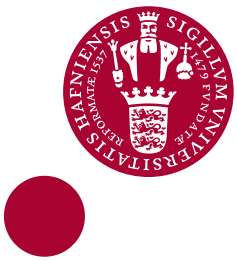

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DYNAMICAL AND STRUCTURAL PROPERTIES OF LIPID MEMBRANES IN RELATION TO LIPOSOMAL DRUG DELIVERY SYSTEMS

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Abstract: The structural and dynamical properties of DPPC liposomes containing lipopolymers (PEG-lipids) and charged DPPS lipids have been studied in relation to the lipid membrane interaction of enzymes and peptides. The results suggest that both the lipid membrane structure and dynamics and in particular the appearance of small-scale lipid structures might be of importance for the activity of membrane associated and liposome degrading enzymes as well as for the membrane interaction of acylated peptides. The combined experimental and simulation results are of relevance for a rational development of peptide loaded liposomal drug delivery systems that become destabilized by membrane degrading phospholipase A_2 enzymes, which are found at elevated concentrations at diseased sites.

Key Words: Lipid Membranes, Phase Transition, Phase Equilibria, Lipid Domain, Phospholipase A_2, PEG-Liposomes, Drug Delivery, Acylated Peptides, Computer Simulation, Fluorescence

INTRODUCTION

A rational development of liposomal drug delivery systems requires insight into the structural and functional biomaterial properties of lipid membranes [1-4]. The overall lipid composition and the interactions between the different lipid components constituting the lipid membrane as well as the thermodynamic conditions imposed by temperature, degree of hydration, and ionic strength are of importance for both the structural and functional behavior of lipid

membranes [3-5]. In particular, it has been demonstrated that a close relationship exists between the lipid membrane microstructure and the functional lipid membrane properties. Examples include the trans-membrane permeability, the stability of liposomes, the interaction of enzymes, peptides, and drugs with lipid membranes as well as the activity of liposome degrading enzymes [5-9]. The lipid membrane microstructure is strongly influenced by the cooperative many-particle membrane behavior, which can lead to the formation of a highly dynamic and heterogeneous lateral membrane structure. The appearance of small-scale lipid structures on length scales of 10-1000 Å is described by a lipid correlation function, which in particular can become large close to phase transitions and critical demixing points of the many-particle lipid membrane [4,10]. A large number of results from both experimental and theoretical investigations of well-defined lipid membrane systems have clearly shown that the cooperative membrane behavior plays an important role for both structural and functional biomaterial membrane properties that furthermore are of relevance for the development and application of liposomes as targeted drug carrier systems [2,5,11].

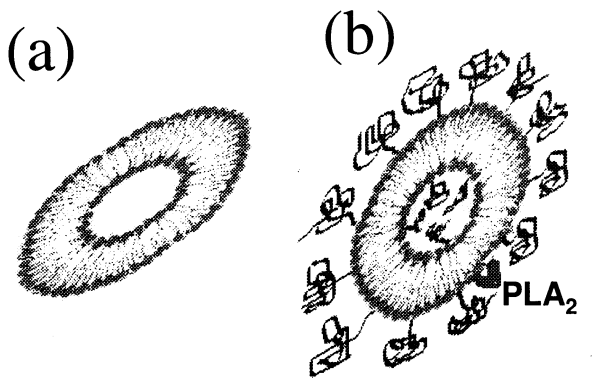

Fig.1. Schematic illustration of a bare phospholipid liposome (a) and a polymer coated PEG-liposome (b) with a phospholipase A_2 enzyme attached to the lipid membrane surface.

In general, the potential use of liposomes for targeted delivery of highly potent although very toxic therapeutic agents to diseased sites has been limited due to a rapid removal of bare liposomal drug carriers as illustrated in Fig. 1a from the blood-stream [2]. However, a significant and advantageous increase in the vascular circulation time can be achieved by incoporating small amounts of synthetic lipopolymers, PEG-lipids, into the liposomes as schematically shown

in Fig. 1b [2,3]. The protective effect induced by the lipopolymers against degradation by, e.g. macrophages in the vascular system is generally understood in terms of a steric barrier induced by the flexible polymer chains attached to the surface of the liposomes. The long-circulating PEG-liposomes, which passively will accumulate in pathological tissue due to leaky capillaries, can be used for targeted transport of conventional water soluble drugs encapsulated into the interior of the liposomes as well as for the potential delivery of small peptide hormones and drugs that associate superficially with the liposome surface [2,12,13]. In the case of amphiphilic peptide drugs which bind to the lipid membrane interface, the hydrophilic-hydrophobic characteristics of both the peptide drugs and the lipid membrane interfacial region are of importance. The strength of the association of conventional and potential peptide drugs with the lipid membrane interfacial region can furthermore be increased by the linkage of a hydrophobic acyl chain anchor to the active compounds [14,15].

LIPID MEMBRANE STRUCTURE AND ENZYME FUNCTION

Results from both experimental and theoretical investigations have shown that the physical properties and phase behavior of the lipid membrane play an important role for the functioning of lipid membrane associated enzymes and proteins [1,2]. A particular interesting example is related to the activity of phospholipase A_2 (PLA_2), which is small water soluble enzyme that is found both in extra- and intracellular compartments of human tissues [8,9,16]. Phospholipase A_2 lipid catalysis involves adsorption of the enzyme to the lipid membrane interface followed by a period of low activity, the so-called lag-time, after which rapid hydrolysis of the phospholipids takes place. Both experimental and theoretical results have demonstrated that the activity of PLA_2 depends strongly on the physical state and microstructural behavior of the lipid membrane substrate [8,9]. In particular, a pronounced temperature and acyl chain length dependent PLA_2 activity is observed in the main phase transition region of phospholipid membranes as shown in Fig. 2 [8,9]. This implies that a strong relationship exists between the enzymatic activity and the lipid membrane microstructure, e.g., the formation of a heterogeneous lateral membrane structure composed of dynamic lipid domains and a network of interfacial regions [4,5,8]. In addition, it has been argued that the sudden change in the lipid membrane composition that takes place due to the fast generation of PLA_2-catalyzed lysolipid and fatty acid hydrolysis products at end of the lag-time, leads to the formation of long-living non-equilibrium small-scale lipid structures [16,17]. Similar non-equilibrium lipid domains have been observed in lipid membrane mixtures after a sudden temperature quench of the lipid membrane from the one-phase fluid region into the two-phase gel-fluid phase coexistence region [18].

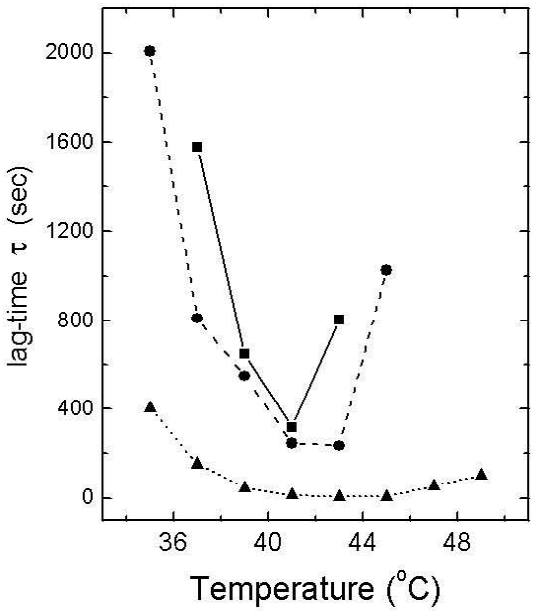

Fig. 2. Phospholipase A_2 lag-time as a function of temperature in the main phase transition region for the hydrolysis of pure DPPC liposomes (solid line) and DPPC liposomes incorporated with 2.5 mol% PE-PEG_{2000} (dashed line) and 5 mol% PE-PEG_{2000} (short dashed line) lipopolymers [21].

Important drug delivery aspects are related to the in vivo destabilization and interaction of lipid membrane degrading enzymes such as PLA_2 with polymer grafted PEG-liposomes. This is of particular interest for the promising use of long circulating PEG-liposomes for targeted transport of encapsulated drugs to pathological tissue where PLA_2 is present at elevated concentrations [19]. A systematic study of the activity of PLA_2 towards polymer coated liposomes has revealed a remarkably lipopolymer concentration dependent increase in the activity of PLA_2 over broad temperature ranges in the main phase transition region as shown in Fig. 2 [20,21]. Such results suggest that the enhanced enzymatic activity of PLA_2 towards polymer grafted liposomes is of importance for the extravascular degradation. Furthermore this non-trivial effect can be used to optimize and target the liposome degradation to take place in pathological tissue where the activity of PLA_2 is significantly increased [19,21].

LIPID MEMBRANE MIXTURES

The lateral organization of the lipids in binary lipid membranes is basically determined by the interactions between the unlike lipids constituting the lipid

membrane. For lipid membrane mixtures, which can undergo phase separation phenomena, the dynamic phase separation process of coexisting phases can give rise to the existence of a highly heterogeneous lateral membrane structure composed of non-equilibrium lipid domains [10,18]. Interestingly, it has been observed that a close structure-function relationship exists between the lipid membrane phase structure of a binary DMPC-DSPC lipid mixture in the gel-fluid phase coexistence region and the activity of PLA_2 [17]. This is manifested as a large increase in the PLA_2 activity in the two-phase region of the DMPC-DSPC lipid membrane as compared to the one-phase gel or fluid regions as shown in Fig. 3 [17,18]. The enhanced enzymatic activity in the two-phase region is most likely related to the existence of long-living non-equilibrium small-scale lipid structures as illustrated in Fig. 4.

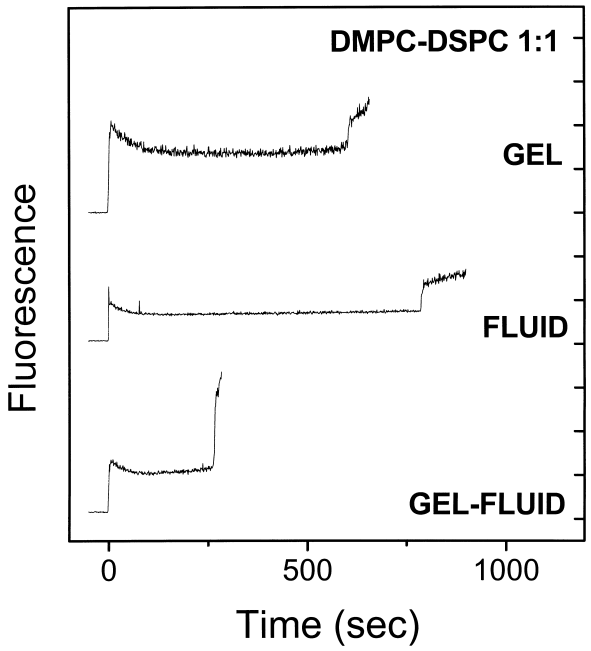

Fig. 3. Characteristic reaction time profiles for phospholipase A_2 hydrolysis of unilamellar DMPC-DSPC liposomes (1:1) in the low-temperature gel region, the high-temperature fluid region, and the gel-fluid phase coexistence region. The PLA_2 hydrolysis reaction is monitored by intrinsic fluorescence from the enzyme. After adding PLA_2 to the equilibrated liposome suspension at t = 0 sec, a characteristic lag-time follows before a sharp change in the fluorescence signals a sudden increase in the catalytic activity of the enzyme [17].

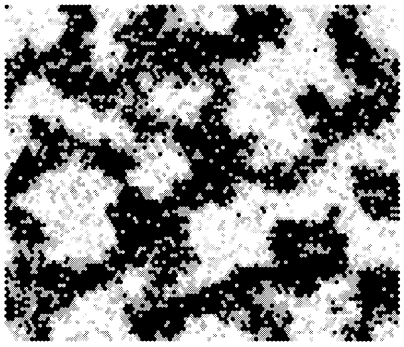

Fig. 4. Snapshot of the simulated non-equilibrium lateral configuration of an equimolar DMPC-DSPC lipid membrane mixture in the gel-fluid phase coexistence region [17,18].

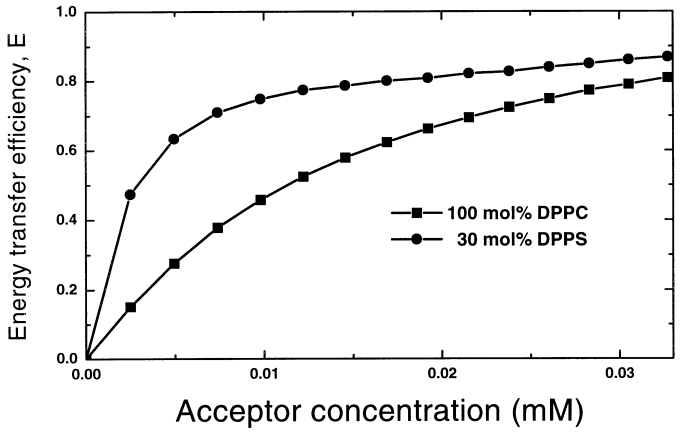

Fig. 5. Fluorescence energy transfer efficiency, E, between tryptophan (donor) in the acylated and cationic peptide and head-group labeled dansyl-PE lipids (acceptor) in the membrane for pure DPPC liposomes and DPPC liposomes containing 30 mol% negatively charged DPPS lipids. The final peptide to lipid ratio is 1:37 [14].

Figure 5 shows how the binding of an acylated model decapeptide is influenced by the lipid composition of a binary DPPS:DPPC lipid membrane mixture [14]. The fluorescence energy transfer efficiency results in Fig. 5 reveal that the acylated and cationic peptide associates with lipid membranes containing negatively charged DPPS lipids (30 mol% DPPS in DPPC) as well as with lipid membranes composed of pure DPPC. However, the slope of the curve for the binary DPPS:DPPC lipid membrane is much steeper indicating a higher binding

affinity towards the two-component DPPS:DPPC lipid membranes [14]. Interestingly, computer simulation results have demonstrated that the mixing properties and the underlying phase diagram of lipid membrane mixture are of importance for the formation of local lipid structures even in one-phase fluid or gel regions which frequently and mistakenly are characterized as homogeneous phases. A preferential interaction of the positively charged peptide with small-scale anionic lipid structures may enhance the peptide binding to the liposome surface and further promote the formation of lipid domains on different time- and length-scales [14,22].

CONCLUSION

The combined theoretical and experimental studies reported above provide information about the many-particle behavior of the lipid membrane that might be of relevance for a deeper understanding of the relationship that exists between the structural and functional biomaterial properties of liposomes [4,5]. In particular, a detailed insight into the influence of lipid composition on structural and functional biomaterial properties of composite liposome systems are of relevance for a rational modification and optimization of liposomes as targeted drug carrier systems. The results presented above might advantageously be used to design and optimize the site-specific in vivo degradation of drug and peptide loaded liposomes to take place at the desired pathological sites where the long-circulating polymer grafted liposomes naturally will accumulate and become degraded by PLA_2 [4,21].

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