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Observation of the main phase transition of dinervonoylphosphocholine giant liposomes by fluorescence microscopy

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Abstract

The phase heterogeneity of giant unilamellar dinervonoylphosphocholine (DNPC) vesicles in the course of the main phase transition was investigated by confocal fluorescence microscopy observing the fluorescence from the membrane incorporated lipid analog, 1-palmitoyl-2-(*N*-4-nitrobenz-2-oxa-1,3-diazol)aminocaproyl-*sn*-glycero-3-phosphocholine (NBDPC). These data were supplemented by differential scanning calorimetry (DSC) of DNPC large unilamellar vesicles (LUV, diameter ~0.1 and 0.2 μm) and multilamellar vesicles (MLV). The present data collected upon cooling reveal a lack of micron-scale gel and fluid phase coexistence in DNPC GUVs above the temperature of 20.5 °C, this temperature corresponding closely to the heat capacity maxima (T_{em}) of DNPC MLVs and LUVs (T_{em} ≈21 °C), measured upon DSC cooling scans. This is in keeping with the model for phospholipid main transition inferred from our previous fluorescence spectroscopy data for DMPC, DPPC, and DNPC LUVs. More specifically, the current experiments provide further support for the phospholipid main transition involving a first-order process, with the characteristic two-phase coexistence converting into an intermediate phase in the proximity of T_{em}. This at least macroscopically homogenous intermediate phase would then transform into the liquid crystalline state by a second-order process, with further increase in acyl chain *trans*→*gauche* isomerization.

Keywords: Giant liposome; Phase transition

1. Introduction

Lipids provide the basic structural framework of all cellular membranes and, characteristically for liquid crystalline materials, exhibit a variety of different phases with connecting transitions [1]. The physical properties and thermodynamic state of the bilayer modulate both the activity and the lateral organization of the contained membrane proteins [2–4], while the proteins also determine in part the ordering of the lipids in the bilayer [5–7]. The molecular mechanisms underlying this dynamic interplay are being intensively investigated (e.g. [8–10]), as lateral heterogeneity and domain formation are known to be involved in several biological processes [11,12]. Biomembranes of eukaryote cells mainly exist in the fluid, liquid disordered state in physiological conditions. Yet, physical changes in the nerve membrane accompanying the prop-

Abbreviations: C_p, excess heat capacity; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DNPC, 1,2-dinervonoyl-*sn*-glycero-3-phosphocholine; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; GUV, giant unilamellar vesicle; Δ*H*, enthalpy change; LUV, large unilamellar vesicle; MLV, multilamellar vesicle; NBDPC, 1-palmitoyl-2-(*N*-4-nitrobenz-2-oxa-1,3-diazol)aminocaproyl-*sn*-glycero-3-phosphocholine; PE, phosphatidylethanolamine; PC, phosphatidylcholine; PPDPC, 1-palmitoyl-2[10-(pyren-1-yl)]decanoyl-*sn*-glycero-3-phosphocholine; *T*, temperature; T_{em}, heat capacity maximum; T_m, main phase transition temperature (corresponding to 50% of the transition enthalpy); X_{lipid}, mole fraction of the indicated lipid

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agation of action potential clearly involve a transient liquid disordered–ordered phospholipid phase change [1,13]. The physical properties and co-operative modes underlying thermal phase behavior of phospholipids also contribute to the established nanoscale lateral heterogeneity [14].

The theory of structural phase transition dynamics of soft materials remains incomplete [15]. The phase diagrams of multicomponent lipid mixtures can be exceedingly complex since, in addition to the different phases possible for single component membranes, there can be solid–solid [16,17], solid–fluid [18], as well as fluid–fluid [19,20] immiscibility. In order to establish a detailed description of the properties (including lateral organization) of lipid mixtures, it is mandatory to understand the mechanism of phase transition in single component membranes.

Dynamic lateral heterogeneity due to coexisting fluctuating gel and liquid crystalline domains accompanies the main transition of phospholipids [21–24]. Upon $T \rightarrow T_m$ the intensity of these fluctuations are enhanced causing the bending elasticity and both lateral (area) and transversal compressibilities to have their maxima at T_m [25–27]. The permeability maximum of bilayers and augmented activity of phospholipases A_2 near T_{em} have been attributed to the length of the phase boundary also having a maximum at T_{em} [28–30]. Many of the previous studies on main phase transition have assumed the heat capacity maximum to be identical to T_m [25,26]. However, transition temperature T_m is, by definition, the melting point where 50% of the transition is completed and it is thus not necessarily identical to the temperature of the heat capacity maximum (T_{em}), particularly in the case of strongly asymmetric C_p peaks [31,32].

Our previous studies on the main transition of phospholipid LUVs suggest that the discontinuities seen in the fluorescence properties may require modification of the existing models describing the phospholipid main transition as a first-order process involving only gel and fluid phases. More specifically, we have recently forwarded a more detailed description of the main transition based on time-resolved fluorescence spectroscopy of DPPC and DNPC [31,32]. In brief, characteristically to a first-order transition, fluid-like domains start to form in the gel phase bilayer upon heating. This fraction of the 'melted' lipids and the length of the interfacial boundary both increase with temperature, i.e. with the progression of the transition. In keeping with the model by Heimburg [33], the fluid-like domains would start to form in the line defects initiated at the corrugations appearing at T_p. Upon approaching T_{em}, the phase boundary seems to disappear, with the formation of an intermediate phase. The latter was suggested to result from the properties of the coexisting fluid-like and gel phases approaching each other as two parallel second-order processes developing with temperature, causing progressively diminishing line tension and hydrophobic mismatch. At T_{em}, fluctuations would be most intense, the entire membrane reaching an intermediate phase and becoming equivalent to the 'boundary'-like lipids. This strongly fluctuating intermediate phase would then transform into the liquid disordered phase as a second-order transition with weak first-order characteristics due to heterophase fluctuations [34], with further increase in acyl chain *trans*→*gauche* isomerization. Upon the completion of this process, the bilayer is in a liquid disordered phase [32,35].

The above model was mainly derived from the behavior of a pyrene-labelled fluorescent phospholipids derivate 1-palmitoyl-2[10-(pyren-1-yl)]decanoyl-*sn*-glycero-3-phospho-choline (PPDPC) in DMPC [36], DPPC [31], and DNPC [32] matrices. Accordingly, it must be emphasized that because of the presence of the fluorophore, we were observing the transition of an 'impure' lipid matrix and the mechanism forwarded applies in strict sense to the bilayer melting in the presence of the contained probes.

In the current study, we addressed the putative intermediate phase by confocal microscopy of dinervonoylphosphocholine (DNPC) giant liposomes using NBD-labeled phosphocholine as the fluorescent probe ($X=0.02$). DNPC contains very long 24:1-*cis*15 chains and thus forms significantly thicker bilayers than DPPC, for instance. We are not aware of DNPC being found in cells and its use as a model was merely dictated by the possibility of being able to study the macroscopic organization of a bilayer undergoing thermal phase transition using fluorescence microscopy. The augmented partitioning of NBD-labelled phosphatidylcholine analogs into the liquid disordered phase has been utilized to observe the coexisting micron-scale phases in the course of the main transition [37,38]. The impact of the fluorescent probe on the main phase transition of the matrix phospholipid was studied by differential scanning calorimetry (DSC).

2. Materials and methods

2.1. Materials

1,2-nervonoyl-*sn*-glycero-3-phosphocholine (DNPC) and 1-palmitoyl-2-(*N*-4-nitrobenz-2-oxa-1,3-diazol)amino-caproyl-*sn*-glycero-3-phosphocholine (NBDPC) were from Avanti Polar Lipids (Alabaster, USA). The concentration of DNPC was determined gravimetrically. In brief, 50 μl aliquot of the lipid in chloroform was transferred with a Hamilton microsyringe onto weighing pans. The solvent was subsequently removed under a gentle flow of nitrogen and keeping the samples under reduced pressure for approx. 1 h before recording the weight of the lipid residue with a high-precision electrobalance (Cahn 2000, Cahn Inc., Cerritos, USA). The concentration of NBDPC was determined by spectroscopy using a molar absorptivity $\varepsilon_{465}=19{,}000$ (in C_2H_5OH). The purity of the lipids was checked by thin layer chromatography on silic acid coated plates (Merck, Darmstadt, Germany) using chloroform/methanol/water (65/25/4, v/v/v) as eluent. An examination of the plates after iodine staining or upon UV-illumination