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Chemistry and Physics of Lipids 135 (2005) 117–129
CPL
CHEMISTRY AND PHYSICS OF LIPIDS
www.elsevier.com/locate/chemphyslip

Effect of grafted PEG on liposome size and on compressibility and packing of lipid bilayer

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Received 16 November 2004; received in revised form 8 February 2005; accepted 8 February 2005
Available online 19 March 2005

Abstract

The aim of this study was to elucidate the effect of various mole percentages (0–25 mol%) of 2000 Da polyethylene glycol–disteroylphosphoethanolamine (PEG–DSPE) in the presence or absence of 40 mol% cholesterol and the effect of degree of saturation of phosphatidylcholine (PC) on the size and the lipid bilayer packing of large unilamellar vesicles (LUV). Egg PC (EPC, unsaturated) LUV and fully hydrogenated soy PC (HSPC, saturated) LUV partial specific volume, specific compressibility, size, and packing parameter (PP) of lipids were characterized by measurements of density, ultrasonic velocity, specific turbidity, and dynamic light scattering. Liposome size and specific turbidity decreased with increase in temperature and PEG–DSPE mol%, except at 7 ± 2 mol%. At this PEG–DSPE mol%, an anomalous peak in liposome size of 15 ± 5 nm was observed. We attribute this effect mainly to the change in the spatial structure of the PEG–DSPE molecule, depending on whether the grafted PEG is in the mushroom or brush configuration. In the mushroom regime, i.e., when the grafted PEG is up to 4 mol% in LUV, the PEG moiety did not affect the additive PP of the lipids in the bilayer, and the PP value of PEG–DSPE is 1.044; while in the brush regime, i.e., when the grafted PEG is higher than 4 mol%, the PP of PEG–DSPE decreases exponentially, reaching the value of 0.487 at 30 mol% of grafted lipopolymer. The specific compressibility and additive PP values for the mixture of matrix lipid (EPC or HSPC), cholesterol, and PEG–DSPE for all liposome compositions investigated reached their maximum at 7 ± 2 mol% PEG–DSPE, the concentration of PEG–DSPE at which the highest biological stability of the LUV is achieved.

Keywords: PEG–DSPE packing parameter; Liposome size; Bulk compressibility

Abbreviations: Chol, cholesterol; DLS, dynamic light scattering; DSPE, distearoylphosphoethanolamine; HSPC, hydrogenated soy phosphatidylcholine; EPC, egg phosphatidylcholine; LD, liquid disordered; LO, liquid ordered; LUV, large unilamellar vesicles; MLV, multilamellar vesicles; PC, phosphatidylcholine; PEG, polyethylene glycol; PP, packing parameter; SO, solid ordered; T_m, phase transition temperature

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0009-3084/$ – see front matter © 2005 Published by Elsevier Ireland Ltd.
doi:10.1016/j.chemphyslip.2005.02.003

1. Introduction

Sterically stabilized liposomes are widely studied as a drug delivery system because of their unique property of long circulation time in the blood stream (Barenholz, 2001; Lasic and Martin, 1995; Lasic and Papahadjopoulous, 1998). Liposome steric stabilization involves physical, chemical, and biological stabilization of the liposomes and retention of their payload. Among phospholipids used as liposome-forming lipids, the most common are phosphatidylcholines (PCs), which form liquid disordered (LD) or solid ordered (SO) phases (Mouristen and Jorgenson, 1994). PCs having acyl chains of various lengths and degrees of saturation can be used as matrix lipids of large unilamellar vesicles (LUV) if their packing parameters (PP), calculated as the cross-sectional area of the hydrophobic moiety divided by the cross-sectional area of the hydrophilic moiety, are in the range of 0.74–1.0 (Kumar, 1991). Another important liposome-bilayer component is cholesterol (Chol), which increases resistance of liposomes to leakage and degradation in vivo (Lasic and Papahadjopoulous, 1998; Samuni et al., 2000, Barenholz, 2004). The presence of Chol in the LUV introduces a new phase in the lipid bilayer, the liquid ordered (LO) phase, and at ∼33 mol% Chol most of the lipid bilayer is in the LO phase (Mouristen and Jorgenson, 1994). Another component, which determines the steric stabilization and the focus of this study, is a lipopolymer composed of the amphipathic polyethylene glycol (PEG) covalently attached to a lipid, e.g., disteroylphosphoethanolamine (DSPE) (Zalipsky and Harris, 1997). It was found that high molecular weight PEG (2000 Da and higher) covalently attached to the lipid headgroup undergoes steric exclusion from the liposome surface (Tirosh et al., 1998) and plays an important role in thermodynamic stability of liposomes by dehydrating the lipid bilayer (Priev et al., 1998). For low molecular weight PEG (<750 Da), there is an insignificant steric stabilization effect (Needham and Kim, 2000). Part of the membrane stabilization by cholesterol is also related to its dehydration effect (Barenholz, 2004; Samuni et al., 2000). In this study, we investigated liposomes having two types of liposome-forming PCs: egg PC (EPC), which is unsaturated (T_m −5 °C) and hydrogenated soy PC (HSPC), which is fully saturated (T_m 52 °C). Bilayers of EPC were in the LD phase at all temperatures used (20–60 °C), while bilayers of HSPC were in the SO phase at temperatures below 52 °C and in the LD phase above this temperature. Addition of Chol to both PCs led to transition to the LO phase.

The aim of this research was to elucidate the effects of various mole percentages of PEG–DSPE, presence of Chol, and the degree of PC saturation on liposome size and compressibility and packing of the lipid bilayer. Two of the components (40 mol% Chol and various mole percentages of PEG–DSPE) were identical, and the third, the matrix PC, was either the "fluid" EPC or the "solid" HSPC. The latter is of special interest as it is the matrix lipid of the liposomal formulation of doxorubicin (Doxil), the widely used, FDA-approved drug in treatment of ovarian and breast cancer (Barenholz, 2001). Fig. 1 presents molecular models of the lipid bilayers studied. Packing and compressibility of lipid bilayer and size of LUV were characterized by density, ultrasonic velocity, dynamic light scattering (DLS), and specific turbidity measurements.

2. Experimental methods

2.1. Lipids

Disteroylphosphoethanolamine–PEG_{2000}, Lot 94-00014, was obtained from Genzyme, Cambridge, MA, USA; egg PC, EPC, (containing mainly 1-palmitoyl-2-oleyl PC, where the acyl chain in position 1 is saturated and in position 2 is unsaturated) and fully hydrogenated soy PC with iodine value 3 (containing mainly disteroyl PC) were obtained from Lipoid, Ludwigshafen, Germany; cholesterol (Chol), Lot 129F8485, was from Sigma, St. Louis, MO, USA. All lipids were ≥98% pure and were used without further purification.

2.2. Liposome preparation

LUV was prepared based on either EPC or HSPC as liposome-forming lipid, with or without 40 mol% Chol, having the desired mol% of PEG–DSPE. All lipids were dissolved in *tert*-butanol, lyophilized (Haran et al., 1993), and resuspended (hydrated) in 10 mM histidine buffer (pH 6.7) to form multilamellar vesicles (MLV). LUV was prepared from the MLV by extrusion through a polycarbonate filter with pore size 100 nm (MacDonald et al., 1991). The LUV extrusions