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Nucleolipid bilayers: A quartz crystal microbalance and neutron reflectometry study

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ABSTRACT

POP-Ade (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidyladenosine) is a biocompatible anionic nucleolipid with the DNA nucleoside, Adenosine, in the polar headgroup. We have studied the affinity of nucleic acids of different contour length, composition and structure toward supported lipid bilayers (SLB) composed of POP-Ade mixed with the zwitterionic phospholipid POPC (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine) using quartz crystal microbalance with dissipation monitoring (QCM-D) and neutron reflectometry (NR). In order to highlight the specificity of the nucleic acid interaction, the results were compared with data obtained for SLB containing the anionic phospholipid POPG (1-palmitoyl-2-oleoyl-*sn*-phosphatidyl-glycerol) replacing POP-Ade. Our results demonstrate that the presence of a nucleobase headgroup provides the bilayers with the ability to bind single stranded nucleic acids in a selective fashion, according to a Watson–Crick pattern. In addition the interaction with double stranded nucleic acids was strengthened. Overall, these findings represent fundamental information for the design of biocompatible DNA vectors with DNA–RNA-based amphiphiles.

1. Introduction

Nucleolipids are synthetic amphiphiles obtained by covalent conjugation of nucleic acid (NA) bases, nucleosides or nucleotides with a lipid moiety [1,2]. Several kinds of nucleolipids have been designed and studied in the past years, prepared by using different synthetic route and/or attachment strategy of the nucleoside–nucleotide unit to the lipid chain, as well as varying the net charge of the polar headgroup, and for the length, saturation and chemical structure of the lipid chain [1]. The building-blocks of DNA–RNA have been employed as single nucleoside or nucleotide moieties, covalently attached to the lipid through functionalization of the ribose/deoxyribose or, more rarely, of the purinic or pyrimidinic heterocycle. Oligonucleotide polar headgroups have been used as molecular zippers between molecular units or self-assembled objects, acting in a responsive way to temperature and ionic strength [3,4]. Moreover, in addition to the naturally occurring nucleic bases, a number of relevant synthetic base analogs with antiviral or antitumor efficacy have been applied to synthesize nucleolipids for which their assembly modulates bioavailability and bioactivity [5,6].

The presence of a bioinspired functional polar headgroup affects the self-assembly properties in aqueous environment, due to additional specific contributions of the polar heads to intermolecular interactions, i.e., π-stacking and hydrogen bonds. Complex architectures and morphologies can be spontaneously built up by exploiting the specificity of Watson–Crick hydrogen bond in the A–T (A–U in RNA) or G–C selective pairing [7]. Moreover, the nucleic moiety can impart smart properties to the surfactant, in terms of binding, reactivity, biocompatibility, recognition, responsiveness, that can be amplified when the molecule is embedded in a self-assembled ordered structure or in a hybrid surfactant/polymer system.

In a recent study 1,2-dilauroyl-*sn*-glycero-3-phosphatidyladenosine nucleolipids inserted in cationic poly(amidoamine) dendrimer layers provided the ability to bind DNA sequences in a specific way, refining the electrostatic attractive interaction that usually drives the formation of DNA/dendrimer polyplexes [8]. The self-assembly structures from nucleolipids have been extensively studied over the past years for applications in the biomedical area, e.g., for DNA arrays [9,10] as

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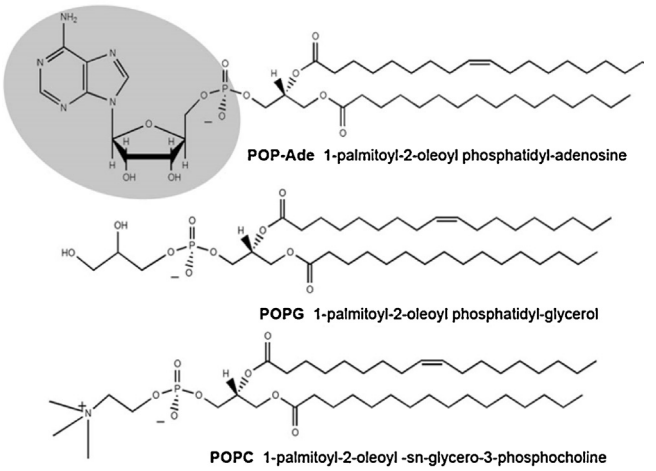

Fig. 1. Chemical structure of the lipids employed to prepare the investigated mixed SLBs: the anionic phospholipids POP-Ade (with a monoanionic adenosine polar headgroup highlighted) and POPG (with a monoanionic glycerol polar headgroup) and the zwitterionic phospholipid POPC.

chemotherapeutic agents and gene delivery vectors [5,11]. Our group has investigated a series of biocompatible phosphatidylnucleosides characterized by different chains and polar headgroups [12,13]. Noticeably, mixed phosphatidylnucleoside self-assemblies (micelles, bilayers, etc.) polar headgroups containing complementary bases exhibit the signatures of interfacial interactions both through π-stacking and specific hydrogen bonds [12]. Nucleolipid assemblies associate with complementary nucleic acid strands both in solution [14] and in liquid–crystalline phases [12]. This ability opens up the possibility for application as DNA vectors for therapeutic and analytic purposes.

The study of lipid-based DNA vectors is still an active field of research, because of the low transfection efficacy and cytotoxicity [15–17] of conventional cationic lipid species, usually employed as vectors for transfection of DNA. Therefore understanding the mechanism of interaction of DNA with nucleolipid assemblies for nucleolipids, which still is not fully understood, is essential for the design of efficient formulations. For instance, the contribution of specific interactions toward a given sequence or secondary structure as well as how the affinity is affected by the NA contour length and the cooperativity of self assembly still remain to be addressed and clarified.

In this study we focus on the nucleolipid POP-Ade (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidyladenosine, see Fig. 1), obtained through substitution of the choline headgroup of POPC (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine), with adenosine. The objective is to reveal how the affinity of nucleic acids toward these nucleolipid bilayers are affected by the nucleic acid length (50-mer or highly polymerized), different composition (complementary, non complementary and random sequence polynucleotides), different structure (single stranded, double stranded). For this purpose we used mixed POP-Ade/POPC liposomal assemblies and studied the formation of lamellar nucleolipoplexes with NA, triggered by addition of Ca^{2+} ions. In order to highlight specific interactions between POP-Ade assemblies and NA, a quartz crystal microbalance with dissipation monitoring (QCM-D) and neutron reflectivity (NR) investigation has been performed on supported lipid bilayers (SLBs) containing either the nucleolipid POP-Ade or the anionic phospholipid POPG (1-palmitoyl-2-oleoyl-*sn*-phosphatidyl-glycerol, see Fig. 1), characterized by the same hydrophobic unit and by the same charged group as POP-Ade, but lacking any specific affinity with NA. This approach has allowed us to single out the first stages of interaction between the lipid membrane and NA, which in solution precede the formation of lipoplexes [18].

2. Materials and methods

2.1. Chemicals

POPC (1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine) and POPG (1-Palmitoyl-2-oleoyl-*sn*-phosphatidyl-glycerol) were purchased from Avanti Polar Lipids (Alabaster, AL). SDS (dodium dodecyl sulphate), HCl, Hepes (4-(2-Hydroxyethyl) piperazine-1-ethanesulonic-acid), $CaCl_2$ and NH_3 (33% aqueous solution) were purchased from Fluka (Buchs, Switzerland); MeOH and $CHCl_3$ were provided by Sigma–Aldrich (St. Louis, MO). PolyU (Polyuridylic Acid), PolyA (Polyadenylic Acid) and DNA (deoxyribonucleic acid sodium salt from calf thymus, type I, highly polymerized) were purchased from Sigma–Aldrich, while oligonucleoltides 50-dA (deoxyriboadenosine) and 50-dT (deoxyribothymidine) were purchased from Atdbio (Southampton, UK). POP-Ade (1-Palmitoyl-2-oleoyl-*sn*-glycero-3- phosphatidyladenosine) was synthesized starting from POPC, Adenosine from Fluka (Buchs, Switzerland) and the enzyme Phospholipase D from Streptomyces sp AA586, purchased from Asahi Chemical Industry (Tokyo, Japan). The synthesis of POP-Ade was performed in a two phase system, according to a modification of the method proposed by Shuto and coworkers [19,20], and obtained as an ammonium salt. Separation from the by-products was achieved by silica gel flash chromatography. Purity was checked by thin-layer chromatography and ^1H NMR.

2.2. Supported lipid bilayers

POP-Ade:POPC 1:1, POPG:POPC 1:1, POP-Ade:POPC 1:4, POPG:POPC 1:4 (mol:mol) lipid mixtures were dissolved in chloroform in glass vials, dried under a stream of nitrogen and further kept under vacuum overnight. Lipid films were then hydrated with the proper volume of a 100 mM NaCl aqueous solution, up to a total lipid concentration of 0.5 mg/ml, vortexed and then tip-sonicated for 15 min to obtain a dispersion of lipid vesicles. For POP-Ade:POPC 1:4, POPG:POPC 1:1 and POPG:POPC 1:4, $CaCl_2$ 10 mM was added to the vesicles' dispersion just before the injection in the measurement chamber, to promote their adhesion to the support and disruption. For the interaction with nucleic acids, DNA–RNA oligonucleotides or polynucleotides were dissolved in pure water and a $CaCl_2$ 0.2 M solution was added dropwise to a 15 mM final Ca^{2+} concentration.

2.3. Preparation of ds-DNA and ss-DNA

Ds-50-mer DNA was prepared by annealing the two complementary 50-mer oligonucleotides, 50-dA and 50-dT. The solution was warmed at 60 °C for 10 min and then cooled down slowly at room temperature. In order to prepare single stranded DNA from Calf Thymus ds-DNA, the solution was thermally denatured by heating at 90 °C for 10 min and then cooled down rapidly by injection into a beaker immersed into an ethanol/ice mixture, according to a procedure described in the literature [21].

2.4. Quartz crystal microbalance with dissipation monitoring

QCM-D experiments were performed on a Q-Sense E4 instrument (Q-Sense, Gothenburg, Sweden) in the Partnership for soft Matter Laboratory (PSCM) of the Institut Laue-Langevin (ILL), Grenoble [22,23], equipped with four flow liquid cells (0.5 ml internal volume), each containing a coated quartz sensor with 4.95 MHz fundamental resonance frequency, mounted horizontally. The active surface of the sensors ($\sim$1 cm^2) was coated with

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