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Polyadenylic acid binding on cationic liposomes doped with the non-ionic nucleolipid Lauroyl Uridine

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ABSTRACT

In this work unilamellar liposomes doped with a novel non-ionic 5′-Uridine-head nucleolipid, Lauroyl Uridine (LU), were prepared and characterized for their ability to interact with the polynucleotide polyadenylic acid (poly-A). Vesicles, were made up of the cationic lipid DOTAP (1,2-Dioleoyl-3-Trimethylammonium-Propane), the zwitterionic lipid DOPE (1,2-Dioleoyl-sn-Glycero-3-Phosphoethanolamine), and the novel amphiphile Lauroyl Uridine. The influence of the non-ionic nucleolipid on essential liposomes properties, such as the structure and net charge was first investigated by a comparative analysis performed on the different lipoplex preparations by means of ζ-potential and size measurements. Both structure and net charge of liposomes were shown to be not modified by the presence of the non-ionic nucleolipid.

The role of the synthetic lipid inserted as anchor in the liposome bilayer in the condensation process between vesicles and the polynucleotide poly-A was then analyzed by UV–vis, Circular Dichroism (CD) and nuclear magnetic resonance (NMR) spectroscopies. The data presented comparative UV–vis analyses that evidenced the occurrence of staking interactions in the poly-A only in LU containing lipoplexes. CD and NMR studies indicated the presence of H-bonding interaction between Lauroyl Uridine containing vesicles and the polynucleotide poly-A. The results presented in this work support a role for Lauroyl Uridine in A-U molecular recognition, thus, suggesting that cationic liposomes doped with the non-ionic nucleolipid Lauroyl Uridine could represent a model system to study molecular interactions among single stranded polynucleotides and lipid anchor bearing the complementary bases.

1. Introduction

Membrane mimetic systems represent the leading model for the study of molecular interactions in a variety of physico-chemical and biological processes. In the recent past the use of colloidal systems such as micelles, vesicles and liposomes, which are able to create compartmentalized environments mimicking the structure of several cell membranes has led to important results in a large number of fields, ranging from fundamental research focused on the physical-chemical properties of the lipid bilayer to applied pharmacology, interested in the development of novel drug delivery systems (see, e.g., Martin et al. [1] and references therein) [2,3]. Among the various biomedical applications, one of the most attractive for the scientific community, concerns the development of novel technologies for direct gene transfer in living cells and thus the development of novel therapies for cancer and genetic diseases [4,5].

A large body of data has been collected in the field of molecular recognition of small molecules at the surface of bilayer vesicles, a central topic in applied biochemistry [6]. For instance, the study of the molecular mechanisms leading to molecular recognition of small molecules at the surface of bilayer vesicles, such as polynucleotide and surfactant, has demonstrated that these molecular interactions can “per se” lead to a DNA collapse process, thus mimicking the condensation process occurring in the nuclei of eukaryotic cells between histones and DNA. In particular, studies on the interactions occurring between self-assembling amphiphiles and biological polyelectrolytes, such as cationic surfactants and DNA, have been fundamental for the comprehension of the phase behaviour of DNA in the presence of oppositely charged surfactants, and the mechanisms underlying the binding of cationic surfactants to DNA [7]. This knowledge is fundamental to understand and thus

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to mimic in biomimetic systems the compaction of DNA, a prerequisite to facilitate the uptake of nucleic acids through the cellular membrane [8]. Along the same line, increasing research efforts are directed toward the development of new liposome formulations in which polynucleotides can be stably encapsulated. Recently, several research groups like ours [9–11] have been focusing on the synthesis of lipids functionalized with nucleobases (nucleolipids), molecules potentially useful for novel liposome formulations.

One of the most appealing applications of the nucleolipids is the formulations of new categories of lipoplexes (complexes between liposomes and nucleic acids), obtained by association of polyadenylic acid with doped liposomes for molecular recognition studies. The DOTAP/DOPE system has been largely utilized to build lipoplexes and it was demonstrated that the electrostatic interactions and the steric effects represent the main driving forces for the association of cationic liposomes and poly-A [12,13]. This work deals with the fabrication of novel cationic liposomal formulations enriched with a functionalized non-ionic nucleolipid Lauroyl Uridine (LU). Since charged molecular chains of poly-A play a key role in determining the interactions with liposomal systems, the introduction of non-ionic 5′-Uridine-head nucleolipid here utilized represents a fundamental characteristic for the formulation of novel cationic lipid-nucleic acid lipoplexes to be employed in molecular recognition studies.

Additionally, the insertion of a Uridine-headed lipid in classical cationic liposomes could provide a novel model system to study molecular interactions among single stranded polynucleotides and lipid anchor bearing the complementary bases. Such models could potentially add new insights in the study of molecular mechanisms involved in membrane fusion after docking steps, in analogy with recent reports centered on synthetic lipid–oligonucleotides interactions and DNA driven sequence-specific hybridization useful to bring membrane surfaces into close proximity and to facilitate membrane fusion [14,15].

In the current paper we report a study on liposomes made up with DOTAP/DOPE and the lipidic pyrimidine based nucleolipid Lauroyl Uridine. After the characterization of the new formed LU liposomes, the study focuses on the interaction between the single stranded polynucleotide poly-A, and LU-free or LU containing liposomes by means of Dynamic light scattering (DLS), ζ-potential, UV–vis, Circular Dicroims (CD) and ^{1}H NMR. The feasibility of producing such novel liposomal formulation for polynucleotide association and the potentiality of the LU lipoplexes for applications in molecular recognition studies are also discussed.

2. Materials and methods

2.1. Materials

The cationic 1,2-Dioleoyl-3-Trimethylammonium-Propane (DOTAP) and the neutral 1,2-Dioleoyl-*sn*-Glycero-3-Phosphoethanolamine (DOPE) lipids were purchased from Avanti Polar Lipids, Inc., Alabaster, AL, and used without further purification. The non-ionic nucleolipid Lauryl Uridine (henceforth indicated as LU, whose structure is reported in Fig. 1) was synthesized and characterized as per a previously reported procedure [9]. Polyadenylic acid (2.3×10^{-3} mol mononucleotide/mg), phosphate Buffer Saline solution (PBS) and deuterium oxide (D_2O) were purchased from Sigma.

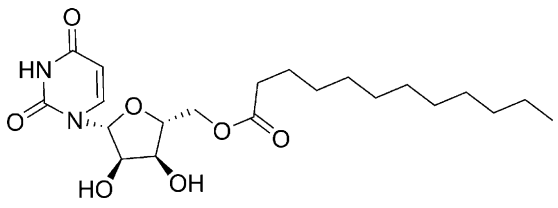

Fig. 1. Lauroyl Uridine chemical structure.

2.2. Sample preparation

Large unilamellar vesicles (LUV) used in this work were made by DOTAP and DOPE at molar ratio 2:1 and different amounts of LU in the following LU/DOTAP ratios: 0, 0.1, 0.2, and 0.3. Mixtures of dry lipid powders were dissolved in chloroform and after solvent evaporation (the samples were left under vacuum over night) the film was swollen and vortexed at room temperature with PBS buffer (pH7.4) to obtain multilamellar vesicles (lipid concentration of 5 mg/ml). Subsequently, samples were subjected to eight freeze/thaw cycles (the suspensions were frozen by immersion into liquid nitrogen and thawed by immersion into 50° water) and then extruded through 100 nm polycarbonate membranes (30 passages). Lipoplexes were prepared adding several amounts of a stock solution of Poly-A to constant volumes of monodispersed suspensions of liposomes. The charge ratios henceforth indicated as −/+ ratio were calculated from the ratio between the negative phosphate groups of poly-A and DOTAP molecules in liposomes. Samples were analyzed not later than 6 h after the preparation.

2.3. DLS and ζ-potential measurements

The measurements of size and ζ-potential were performed using a Zetasizer ZS Nano (Malvern, Malvern, UK). For the size determination the light scattering was detected at an angle of 90° with a laser He–Ne operating at the wavelength of 633 nm. The working temperature was maintained at 25 °C with a peltier element integrated in the apparatus. DLS autocorrelation functions of the scattered light intensity were carried out with DTS 5.0 software provided by the manufacturer, which allowed to determine the distribution of the scattered intensity versus the hydrodynamic diameters. For the measurement of ζ-potential the electrophoretic mobility of the aggregates was determined by laser Doppler velocimetry. The samples were placed in dedicated disposable capillary cells. The cells were calibrated before each set of measurements with a latex standard solution (-50 ± 5 mV). The ζ-potential values were calculated by the Smoluchowski approximation of Henry's equation.

$$U_e = \frac{2\varepsilon k\zeta}{3\eta} \quad (1)$$

were U_e is the electrophoretic mobility, ζ is the zeta potential, ε is the medium dielectric constant, η the viscosity of the solution and $k = 1.5$ (model based constant for salt concentrations higher than 1 mM Smoluchowski 1921). Because of the high conductivity values of the suspensions the measurements were performed with a d.d.p. of 10 V.

2.4. UV–vis, CD, ^{1}H NMR spectroscopies

The UV–vis spectra of the liposomes were collected with a Shimadzu UV-1601 Spectrophotometer in the 200–800 range, at 25 °C, using a quartz cell with a path length of 1 cm. For the hypochromic effect measurements, since the spectra of liposomes are affected by non-negligible contribution due to the scattering of the aggregates, Rayleigh scattering of liposomes was subtracted by fitting a power-law curve to the range of spectrum where absorption from nucleic bases is absent (320 and 500 nm) [16]:

$$A' = K_0\lambda^{-k} \quad (2)$$

where λ is the wavelength in the range defined above, k (for Rayleigh scatterers $k \approx 4$) and K_0 are adjustable parameters. Then, the calculated scattering curve has been extrapolated up to 220 nm