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Biophysical properties of cationic lipophosphoramidates: Vesicle morphology, bilayer hydration and dynamics

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ABSTRACT

Cationic lipids are used to deliver genetic material to living cells. Their proper biophysical characterization is needed in order to design and control this process. In the present work we characterize some properties of recently synthetized cationic lipophosphoramidates. The studied compounds share the same structure of their hydrophobic backbone, but differ in their hydrophilic cationic headgroup, which is formed by a trimethylammonium, a trimethylarsonium or a dicationic moiety. Dynamic light scattering and cryo-transmission electron microscopy proves that the studied lipophosphoramidates create stable unilamellar vesicles. Fluorescence of polarity probe, Laurdan, analyzed using time-dependent fluorescence shift method (TDFS) and generalized polarization (GP) gives important information about the phase, hydration and dynamics of the lipophosphoramidate bilayers. While all of the compounds produced lipid bilayers that were sufficiently fluid for their potential application in gene therapy, their polarity/hydration and mobility was lower than for the standard cationic lipid – DOTAP. Mixing cationic lipophosphoramidates with DOPC helps to reduce this difference. The structure of the cationic headgroup has an important and complex influence on bilayer hydration and mobility. Both TDFS and GP methods are suitable for the characterization of cationic amphiphiles and can be used for screening of the newly synthesized compounds.

1. Introduction

Gene and antisense therapies are proposed to cure cancer, neurodegenerative, cardiovascular, infectious and monogenic diseases. Unfortunately, the lack of techniques that would allow safe and effective transfer of genetic material to target cells (transfection) limits the number of clinical trials in this field [1]. Viruses that evolved to transfect cells very efficiently were often modified and successfully used as gene vectors (carriers) both in vitro and in vivo; however, their application is limited by their relatively low carrying capacity. More importantly, their side effects in patients (e.g. immune response) are difficult to control, which can have fatal consequences [2]. Non-viral vectors, that are mostly based on cationic lipids or polymers, are much safer, cheaper, relatively stable to storage, and their composition can be easily tuned [1,3]. They are also not free from drawbacks though. The main problem is their limited transfection efficiency. They can also suffer from poor biodegradability and/or toxicity. The optimization of the non-viral gene delivery is thus of prime importance.

An ideal gene vector should: (1) protect nucleic acids against degradation in blood, target tissue, and along the administration route (e.g. in stomach in the case of oral administration), (2) pass cellular membrane of a target cell, and (3) release its content to the correct site inside the cell. At the same time, it should be safe to the patient and easy to use. After years of studies in this field (since the introduction of lipid-based transfection by Felgner et al. in 1987 [4]) it seems that development of a universal gene delivery system is not feasible. One should rather focus on designing application-specific multicomponent constructs targeted to certain cells. This is why a number of techniques, new synthetic lipids, and polymers have been tested [1,5–9]. Many of them are also used by cell biologists, who search for new tools to study more biologically relevant yet harder-to-transfect cell lines.

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Lipophosphonates synthesized in our laboratory [10,11] are one of the promising families of cationic amphiphiles designed for gene delivery. Their evaluation as DNA carriers revealed their enhanced efficiency, in the course of both in vitro [12,13] and in vivo [14] transfection assays, often associated with their low toxicity. The introduction of P-N based-linker gave rise to lipophosphoramidates [15,16]. This type of cationic lipid is more easily synthesized thanks to an Atherton-Todd reaction [17,18], their biodegradability is likely improved due to the replacement of a P—C bond by a P—N bond (this linker could be hydrolyzed by enzymes e.g. phosphoramidase-II [19]), and the construction is highly modular allowing us to produce fluorescent lipids [20,21] or antibacterial agents [22].

Cationic lipids, like the most well-known DOTAP [23] or the lipophosphoramidates studied herein, interact electrostatically with nucleic acids condensing them and forming so-called lipoplexes [4]. Lipoplex structure, distribution within the body, transfection efficiency and side effects strongly depend on the lipid composition [24–29]. Thus, to understand the complexation of the nucleic acid with the carrier and the influence of the later on the transfection process a detailed physicochemical characterization of the complex as well as its components is beneficial [30–33].

In this work, we chose to evaluate the influence of the type of polar head of lipophosphoramidate on the properties of liposomal membranes created from either a pure compound or its mixture with zwitterionic dioleoylphosphatidylcholine. We believe that the cationic lipid head group to large extend governs its interactions with nucleic acid and with the lipid membrane of the target cell. The properties of the lipophosphoramidates are compared with those of DOTAP. Lipophosphoramidates with various backbone structures have been also recently characterized in detailed in terms of their physicochemical properties, DNA condensation and transfection efficiency in vitro [32,34,35]. Introduction of double bonds in the backbone chains increased fluidity of the lipid bilayer. Although for some applications this increased fluidity is beneficial [36], no direct link between fluidity and fusion efficiency or DNA condensation was found [32]. All of the herein studied lipids possess the same hydrophobic backbone composed of two oleic chains to ensure sufficient membrane fluidity in the temperature range used. Created liposomes were characterized in terms of their morphology using dynamic light scattering and cryo-electron microscopy. Lipid bilayer hydration and local dynamics of lipids were studied using steady-state and time resolved fluorescence of Laurdan. The obtained results are analyzed using time-dependent fluorescence shift method, generalized polarization and fluorescence anisotropy.

2. Experimental

2.1. Materials

1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) and 1, 2-dioleoyl-3-methylammoniumpropane (DOTAP) were purchased from Avanti Polar Lipids, Inc. (Alabaster, USA). 6-dodecanoyl-2-dimethylaminonaphtalene (Laurdan) was obtained from Molecular Probes (Eugene, USA). HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) was purchased from Fluka (Buchs, Switzerland). Buffer was dissolved in Mili Q water (Milipore, USA). Organic solvents of spectroscopic grade were supplied by Merck (Darmstadt, Germany). All chemicals were used without further purification.

Lipophosphoramidates were synthesized as previously described: dioleoylphosphatidyl-2-aminoethyltrimethylarsonium iodide ($DOHAs^+$) [15,36], 3-(*O*,*O*-dioleylphosphoramidoyl)-propyltrimetylammonium iodide ($DOHN^+$) [15,37], 3-(*O*,*O*-dioleylphosphoramidoyl)-(trimetylammoniumpropyl)-dimethylammonium dibromide ($DOHN^+_2$) [38], 3-(*O*,*O*-dioleylphosphoramidoyl)-*N*-methylpropyltrimetylammonium iodide ($DOMeN^+$) [37]. Chemical structures of the studied lipids are depicted in Fig. 1.

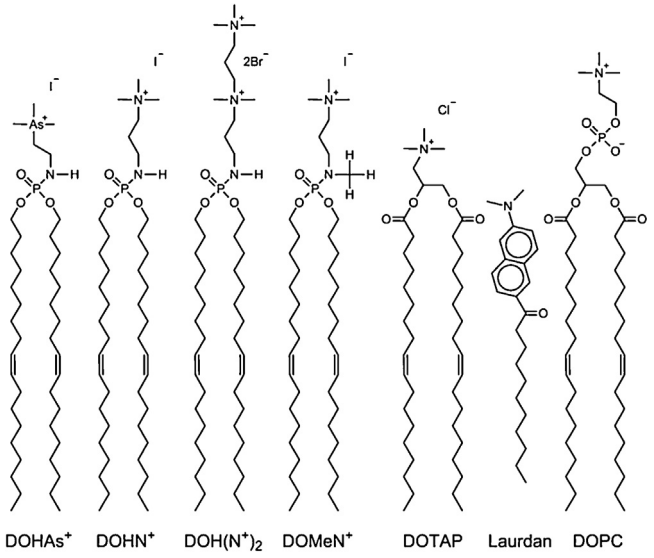

Fig. 1. Chemical structures of the studied cationic lipophosphoramidates together with DOTAP, DOPC, and fluorescence probe Laurdan (positioned according to [49]).

2.2. Preparation of lipid vesicles

An aliquot of a stock solution of lipids in chloroform at 5 mg/mL together with methanol solution of Laurdan was evaporated under a nitrogen stream for 10 min and then under reduced pressure for 1.5 h. The final probe to lipid ratio was 1:100 (mol:mol). 10 mM HEPES buffer pH 7.4 with 150 mM NaCl was added and the samples were kept at 4 °C for at least 1 night for hydration. Then the samples were strongly vortexed and extruded at 25 °C through 100 nm pore-diameter filters (Avestin, Ottawa, Canada; at least 50 passages) in order to obtain LUV dispersion. The final lipid concentration in the liposomal dispersion was 1 mM.

2.3. Dynamic light scattering (DLS)

Mean particle diameters of liposomes were measured by Dynamic Light Scattering (DLS) using a 3000 Zetasizer (Malvern Instruments Ltd., Worcestershire, UK). After appropriate dilution samples were transferred to 1 cm UV grade poly(methyl methacrylate) cuvettes (Kartell, Noviglio, Italy) and equilibrated for 5 min at 25 °C before each measurement. DLS setup (Zetasizer Nano ZS, Worcestershire, UK) consisted of He-Ne laser 532 nm and an avalanche photodiode detector. The scattering intensity was collected at the angle of 173°. The mean vesicle size was obtained using cumulant analysis implemented in Zetasizer Software 6.2 (Malvern Instruments Ltd., Worcestershire, UK).

2.4. Cryo-transmission electron microscopy (cryo-TEM)

In order to prepare vitrified samples of lipid vesicles, we used the Vitrobot (FEI, The Netherlands). Samples (3 μL of a suspension) were applied on the side of glow-discharged 300 mesh copper grids coated with lacey carbon film (SPI, USA) inside the Vitrobot chamber at room temperature and 100% humidity. Grids were blotted for 2–3 s at zero blot offset, plunged into liquid ethane and immediately transferred into liquid nitrogen. Samples in vitrified state were observed the same day in FEI TECNAI G2 20 electron micro-