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Oligonucleotides and polynucleotides condensation onto liposome surface: Effects of the base and of the nucleotide length

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ABSTRACT

The association behavior of different nucleic acids with cationic liposomes has been monitored, in order to find out how the polymer length, the type of base and the charge density affect the lipoplex formation. In particular the associative features displayed by the homopolymer 20-mer of adenine, Oligo (dA), of timine, Oligo (dT), and of guanine, Oligo (dG), were compared to understand the role of the base. The effects of the nucleic acid length and of the charge density were evaluated taking account of the association of the polyadenylic acid and of the DNA onto the liposomes. The results show that the homopolymer Oligo (dG) is able to interact with the cationic liposomes to the same extent as DNA, in spite of the fact that Oligo (dG) is a short polymer made of 20 residues and DNA is a longer and dual strand polymer having a higher charge density.

1. Introduction

Among the drug delivery systems, liposome formulations are considered one of the safest types of carrier [1]. Generally, owing to the anionic nature of DNA, cationic and neutral lipids are used for gene delivery, while the use of anionic liposomes is limited to the delivery of other therapeutic macromolecules. Although several efforts have been made to use neutral or negatively charged liposomes for gene delivery, the limited efficiency of plasmid–DNA encapsulation and consequently the low levels of transfection encouraged researchers to focus their attention on cationic liposomes [2–5]. In the last years, a large number of studies have dealt with the present issue by means of specific strategies like the molecular recognition [4–14].

Cationic liposomes are able to form complexes with nucleic acids and are still one of the most promising non-viral systems in the gene therapy field [15,16]. On the one side, the role of the positively charged head group is well recognized as crucial for binding and complexation of the anionic phosphate groups of the nucleic acid [8,9,17,18]; while, on the other side, the hydrophobic contribution is not entirely understood, even though it has been observed to affect the interactions between lipid aliphatic tails and DNA [19]. The phenomenology of polyanion–cationic liposome interaction is relatively intricate, depending on a number of parameters such as the charge density on the particle surface and on the polyelectrolyte chain, the flexibility of the polyelectrolyte backbone and the physical–chemical properties of the medium [20–22]. Lately, the role played by the charge density of lipoplexes has been studied in detail [23]; on the contrary the effect of the counterion that is supposed to play a key role in the organization of lipoplexes is still a matter of investigation [24,25].

The electrostatic attraction between cationic liposomes and nucleic acids is the main force driving their association but other forces are also involved in the condensation process [20]. In order to describe this sort of interaction we should consider other factors like the charge density, the polymer length and the hydrophobic contribution coming from the lipid-base binding which have been the subject of more debate in recent years [19]. Furthermore, the ability of G-rich region of polynucleotides to self organize in the so-called G-quadruplexes should be taken into account. In fact, it is well known that besides the base paring A-T and G-C in the double helix structure of the DNA, other alternative base paring are able to promote the formation of triple stranded and four stranded structures [26].

In this respect the impact of zeta potential (ζ-potential) on the physical properties of the lipoplexes is extremely meaningful. The

Abbreviations: DOTAP, 1,2-dioleoyl-3-trimethylammonium-propane; DOPE, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine; DNA, deoxyribonucleic acid; DLS, dynamic light scattering.

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ζ-potential is generated when charged particles are dispersed in a medium and results in the formation of an electric potential between the surface of the particle and the medium [27]. This event depends on the surface charge of the particle that attracts counterions, thus forming a double layer where the first layer contains firmly attached ions which are directly adsorbed to the surface and is called Stern-layer. This layer is surrounded by a second layer, the diffusive layer, where the ions are less associated with the surface. The potential between the diffuse layer and the medium is called the ζ-potential [28,29]. The interaction of nucleic acids and cationic liposomes is driven by the electrostatic adsorption of charged macromolecules onto an oppositely charged surface. This issue has recently been addressed by several authors [30–32].

The result of the adsorption of polyanions onto liposomes generates a surface charge distribution that could be homogeneous or not depending on several factors such as the concentration, the length or the type of polyanion, the presence of a particular kind of nucleobase and the charge density of either the polyanion or the liposome. The effectiveness of the condensation is dramatically correlated with the size of the complex formed. Generally, the result of a complete coverage of the liposome surface by the anion is able to reverse the surface charge thus allowing the formation of small complexes held in the distance thanks to the surface repulsion. In opposition, when the liposome surface is partially covered by the anion, the attraction between the opposite microdomains, positive and negative domains, leads the complex formed to interact electrostastically evolving into bigger aggregates [4,5,33].

In the present investigation we have dealt with the association behavior of lipoplexes made by oligo and polynucleotides with cationic liposomes. The formation of DNA–cationic lipids complexes is still matter of investigation [15], thus there is still room for the development of a deeper search. For this purpose we have compared the behavior of different linear polyelectrolytes, differing for length, composition, and charge density in their interaction with charged cationic liposomes. The target we intend to meet is the understanding of the role played by the single nucleic base in the condensation process. The evolution of size and surface charge of the complexes formed between the polyanions (nucleic acids) and the cationic liposome have been monitored as the anionic molecules were added to a fixed concentration of liposome.

2. Materials and methods

2.1. Materials

The cationic 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), the neutral 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) were purchased from Avanti Polar Lipids, Inc. Alabaster, AL, and used without further purification. Oligonucleotide sequences, Oligo (dA)-20mer (M.W. 6202), Oligo (dT)-20mer (M.W. 6022), Oligo (dG)-20mer (M.W. 6447) were custom-made by Sigma Genosys. Polyadenylic acid (2.3×10^{-3} mol mononucleotide/mg), sperm salmon deoxyribonucleic acid (DNA) (average M.W.1.3×10^6 Da; 2000 bp), phosphate buffer saline solution, PBS (containing 10 mM phosphate buffer, 2.7 mM potassium chloride and 137 mM sodium chloride) were purchased from Sigma–Aldrich.

2.2. Sample preparation

Large unilamellar vesicles (LUV) were made by DOTAP and DOPE at molar ratio 1:1. Mixtures of dry lipid powder were dissolved in chloroform and after solvent evaporation (the samples were left under vacuum overnight) the film was swollen and vortexed at room temperature with PBS buffer at pH 7.4 to obtain multilamellar vesicles (lipid concentration of 5 mg/ml). Subsequently, samples were subjected to eight freeze/thaw cycles (the suspensions were frozen by dipping the test tubes into liquid nitrogen and thawed into 50 °C water-bath) and then extruded through 100 nm polycarbonate membranes (30 passages). Lipoplexes were prepared adding several amounts of stock solution of nucleic acids to constant volumes of suspensions of monodispersed liposomes. The charge ratios −/+ were calculated from the ratio between the moles of negative phosphate groups of the nucleotides (oligonucleotides, polynucleotides and ds-DNA) and the moles of DOTAP in liposomes. In detail, the moles of negatively charged Oligo (dA), Oligo (dT) and Oligo (dG) were calculated from the data provided by the manufacturer. The polymers were indeed dispersed in PBS buffer in order to have a stock solution with a concentration of 2 mM. The negative charges of the polyadenylic acid were calculated considering the product information: 2.3 μmol of mononucleotide in 1 mg of polymer. The stock solution prepared in PBS had a concentration of 2.3 mM. The moles of phosphate groups on ds-DNA were estimated considering the characteristics of the polynucleotide average M.W. 1.3×10^6 Da, 2000 bp and an average M.W. per mononucleotide unit of 330 Da. The concentration of phosphate groups of the stock solution of ds-DNA was 1 mM.

2.3. DLS and zeta potential

The measurements of size and ζ-potential were performed using a Zetasizer ZS Nano (Malvern, Malvern, UK). For the size determination the scattering of light was detected at an angle of 90° with a laser He–Ne operating at the wavelength of 633 nm. The working temperature was kept constant 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 (Eq. (1)) [34]:

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

where U_e is the electrophoretic mobility, ζ is the zeta potential, ε is the medium dielectric constant, η is the viscosity of the solution, which was considered as a constant value, according to the manufacturer instruction, because the sample concentration was lower than 0.1% (w/w) and $k = 1.5$ (model based constant for salt concentrations higher than 1 mM) according to Smoluchowski approximation. Because of the high conductivity values of the suspensions the measurements were performed with a voltage of 10 V.

3. Results and discussion

3.1. ζ-Potential outlook

ζ-Potential has been used to measure the electric charge on the liposome surfaces, a parameter that affects the stability of the formulation [35,36]. In general the stability of a colloid system depends on both the repulsive electrostatic forces and the attractive van der Waals forces. The energy barrier provided by the electrostatic repulsions should be large enough in order to shield the particles from coalescence processes thus preventing the occurrence of aggregation phenomena. When the repulsive energy is not adequately strong the particles are prone to aggregation. This