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Structure and internal organization of overcharged cationic-lipid/peptide/DNA self-assembly complexes

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ABSTRACT

The combination of cationic lipids with cationic peptides and DNA vectors can produce synergistic effects in gene delivery to eukaryotic cells. Binary complexes of cationic lipids with DNA are well-studied whereas little information is available about the structure of the ternary lipid/peptide/DNA (LPD) complexes and mechanisms defining DNA protection and delivery. Here we use synchrotron small angle X-ray scattering and dynamic light scattering zeta-potential measurements to determine structure and the net charge of supramolecular aggregates of complexes in mixtures of plasmid DNA, cationic liposomes formed from DOTAP, plus a linear cationic ε-oligolysine with the pendant α-amino acids Leu-Tyr-Arg (LYR), ε-(LYR)K10. These ternary complexes display multilamellar structures with relatively constant separation between DOTAP bilayers, accommodating a hydrated monolayer of parallel DNA rods. The DNA–DNA distance in the complexes varies as a function of the net positive to negative (lipid + peptide)/DNA charge ratio. An explanation for the observed dependence of DNA–DNA distance on charge ratio was proposed based on general polyelectrolyte properties of non-stoichiometric polycation–DNA mixtures.

1. Introduction

Synthetic vector-based gene delivery relying on chemical principles has gained much interest for its safety, biocompatibility and potential for large-scale production [1,2]. Its development offers the potential to circumvent the limitations of viral gene delivery in both basic research laboratories and clinical settings [3]. Phospholipids and peptides are frequently used gene delivery materials [4]. Recently, ternary lipid/peptide/DNA (LPD) complexes have been developed as a promising gene transfer tool [1,5,6]. The improved gene delivery performance of the LPD complex compared to the corresponding binary systems was observed in many cases and studies of the mechanism behind this effect has received much attention [7–12].

Our previous finding suggested that a novel class of synthetic cationic peptides, namely branched ε-oligolysines, based on linear ε-oligolysines with short α-amino acid chains attached to each residue of the main chain (Fig. 1), can cover DNA on the surface of ternary multilamellar complexes of cationic lipids (DOTAP)/ε-oligolysine/DNA [12]. Caracciolo et al. reported that DNA was found at the surface of lipid/DNA particles (lipoplex), while this was not found for lipid/protamine/DNA complexes [11]. In studies of lipoplexes, Oberle et al. found that the imperfection in the lipid/DNA complex formation leads to a partial exposure of plasmid DNA on the surface of the particles [13]. The partial DNA exposure may cause complex clustering and interact with cellular components, thereby inhibiting transfection. Huebner et al. observed this phenomenon for lipoplexes using cryoelectron microscopy as a multilamellar structure with unclosed bilayers at high charge ratio lipid/DNA (lipoplex with open rims of the outer lamellae at low L/D [14]), suggesting that imperfections in lipoplexes occur irrespective of the charge ratio.

Liposomes from cationic phospholipids condense DNA, resulting in a self-assembled complex with ordered structures, which were extensively studied by synchrotron small angle X-ray scattering (SAXS) and shown to influence transfection activities [15–19]. Safinya and co-workers reported that DOTAP/DNA complexes exhibit a multilamellar structure [15]. Inside the complexes, the periodic distance of the multilamellar structure suggested a monolayer of hydrated DNA intercalated between lipid bilayers [15]. Within the layer, the DNA chains are orderly arranged in parallel and give rise to a DNA–DNA correlation peak (q_{DNA}) in SAXS spectra [15]. In our previous study, we found self-assembly of the three components (liposome prepared from DOTAP, ε-oligolysine and DNA) to ordered multilamellar complexes as illustrated in Fig. 2. In the cited work, the charge ratio of lipid DOTAP to DNA (L/D) was fixed and equal to 2 and the charge

Abbreviations: CR, charge ratio; $CR_{+/-}$, charge ratio of positive to negative; d_{DNA}, interaxial DNA–DNA distance; $d_{interlayer}$, interlayer spacing; DLS, dynamic light scattering; DOPE, 1,2-di-(9Z-octadecenoyl)-sn-glycero-3-phosphoethanolamine; DOTAP, 1,2-dioleoyl-3-trimethylammonium-propane; HBS, Hepes-buffered saline; L/D, charge ratio of lipids to DNA; Lipoplex, lipid/DNA complexes; LPD, lipid/peptide/DNA complexes; ε-(LYR)K10: α-substituted ε-K10, the amino acid letters in the brackets denote the substitution groups; P/D, charge ratio of peptides to DNA; SAXS, small-angle X-ray scattering

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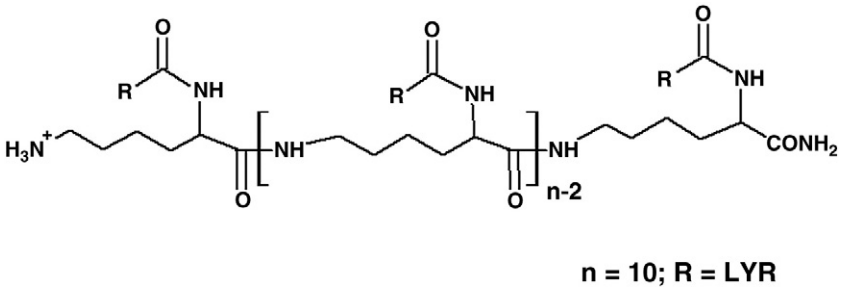

Fig. 1. Chemical structure of α-substituted derivatives of ε-oligolysine ε-(LYR)K10 studied in this work.

ratio of ε-oligolysine to DNA (P/D) was varied in the range of 0.3–5, resulting in overcharged complexes [12]. Peptides distributed in the DNA layer are associated with DNA and contribute to DNA charge neutralization [12]. The structural information was found to be consistent with the observed synergistic effect of DOTAP and ε-oligolysines improving DNA delivery compared to the binary lipid/DNA system [12]. This finding gave an insight about the internal nanostructure of the transfection complex. However, the understanding of the physico-chemical mechanism in formation of the resulting overcharged complexes is still limited.

The dependence of DNA–DNA distance, d_{DNA}, on the positive to negative charge ratio (L/D) was reported for lipid/linear DNA complexes, where either cationic lipid or its mixture with neutral lipids was used [17]. In the present work, the influence of the lipid and peptide composition on the structure of LPD complexes, DOTAP/ε-oligolysine/DNA mixtures with variation of the total lipid + peptide/DNA charge ratio ($CR_{+/-}$) from 1 to 7, was examined by synchrotron small angle X-ray scattering (SAXS). Since both lipids and peptides provide positive charges in the ternary complex, various lipid–peptide mixture compositions are tested, as indicated by the charge ratio lipid/DNA (L/D) and peptide/DNA (P/D). It was found that the DNA–DNA distance in the lamellar structure and the surface charge density (measured by dynamic light scattering zeta potential measurements) of the particles is mainly dependent on the total charge ratio $CR_{+/-}$. For a given $CR_{+/-}$, the DNA–DNA distance is insensitive to the changes of L/D and P/D. It is suggested that the ordered multilamellar structure is maintained by lipid–DNA interaction, whereas the internal organization of the hydrated DNA in the LPD complexes is sensitive to the total balance of positive and negative charges. This implies that peptide molecules are present inside the multilamellar stacks between DNA molecules and that they also influencing the surface charge of the LPD aggregates by association at the surface of the particles. We suggest a simple explanation for the observed variation of d_{DNA} with $CR_{+/-}$ based on an electrostatic analysis of the thermodynamics of non-stoichiometric polycation–polyanion mixtures. This study gives important general information about the physico-chemical mechanism of phospholipid–peptide–DNA self-assembly and contributes to the understanding of the biophysical properties of LPD systems, which aids in further development of these as non-viral gene delivery vehicles.

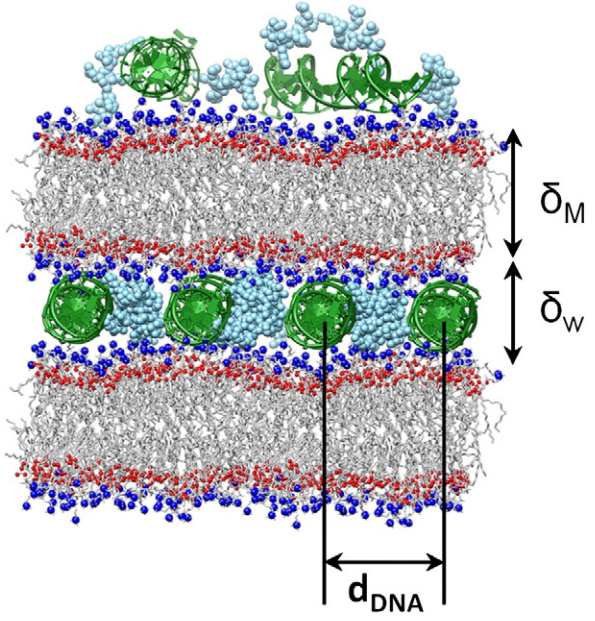

Fig. 2. Schematic picture illustrating the lamellar phase of DOTAP/ε-peptide/DNA complexes, with alternating lipid bilayers and DNA monolayers. Peptides are arranged between DNA chains within the DNA monolayers. The DNA interaxial spacing is dDNA. The interlayer spacing is $d = \delta_m + \delta_w$. The top layer of DNA and peptides associated with the lipid surface illustrates the surface of the overcharged aggregates.

2. Materials and methods

2.1. Materials

2.1.1. DNA

The plasmid pEGFP-N1 (4.7 kbp) encoding the green fluorescent protein GFP was used. The plasmid was amplified in the *Escherichia coli* DH5α strain and extracted by alkaline lysis method. The DNA used in present work was the same as applied for transfection and biophysical studies in our earlier work [10,12].

2.1.2. Peptides

As cationic peptide we used an ε-oligolysine with degree of polymerization 10 and α-amino acid triplet, Leu-Tyr-Arg (LYR) attached to each of the α-amino group of ε-oligolysine, ε-(LYR)K10, (Fig. 1). ε-(LYR)K10 was synthesized using solid-phase peptide synthesis as described in detail in earlier work [10]. The peptide stock solutions (5–25 mg/ml) were prepared in sterile, double-distilled water from lyophilized trifluoroacetic salt. In solutions of neutral pH, amino groups of the ε-(LYR)K10 should be fully protonated and the peptide carries a charge +21e.

2.1.3. Lipids

DOTAP (1,2-dioleoyl-3-trimethylammonium-propane, chloride salt) and DOPE (1,2-di-(9Z-octadecenoyl)-sn-glycero-3-phosphoethanolamine, chloroform) used in SAXS sample preparation were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL, USA). DOTAP solution in chloroform was dried under vacuum at room temperature for 1 h and dissolved in deionized water to concentration 25 mg/ml. Resuspended DOTAP was either extruded by LiposoFast-Basic with 100 nm polycarbonate membrane (Avestin, Inc., Canada) or sonicated with 1 s pulse of 30% amplitude for 10–15 min to clarity by tip sonicator Sonics vibra cell™ (Sonics & Materials Inc., USA). The freshly prepared DOTAP liposomes (mean hydrodynamic diameter of ~100 nm and polydispersity of ~0.1 according to DLS measurement) were stored at room temperature. Liposomes comprised of binary mixture of DOTAP and DOPE are prepared as abovementioned after mixing DOTAP and DOPE chloroform solution at ϕ_{DOPE} (weight fraction) = 0.75.