Stability of oligodeoxynucleotide complexes with cationic phospholipid and peptide carriers

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ABSTRACT

The design of efficient cationic lipid and peptide nanocarriers for oligodeoxynucleotide (ODN) delivery into cells requires precise understanding and control of their physical properties. In this work we used X-ray diffraction, fluorescence resonance energy transfer (FRET), dynamic light scattering (DLS) and zeta-potential measurements to characterize the stability of the nanoparticles formed by ODN with cationic phospholipids and with a native cationic peptide, protamine. The most important findings can be summarized as follows:
1) ODNs form stable complexes with protamine and cationic phospholipids at salt concentrations below ~0.5 and ~0.3 M NaCl, respectively.
2) The ODN lipoplexes are typified by tightly packed lamellar phases and disordered ODN arrangement between the lipid bilayers.
3) ODN/protamine complexes with positive zeta-potentials gradually increase in size in the course of hours and days, while the size of nanoparticles with negative zeta-potentials remains constant.
4) There are significant differences between ODN/lipid and ODN/protamine particle size distributions. The former displayed a characteristic maximum at cationic/anionic 1:1 charge ratio, while the latter were not influenced by variations of the ODN/protamine ratio.

1. INTRODUCTION

Cationic lipid and peptide compounds are commonly used as carriers for DNA delivery into cells. Similarly to gene transfection, the use of cationic carriers for the delivery of oligomeric DNA and RNA with important regulatory functions is also of high current interest. However, due to large electrostatic differences in the binding of oligomeric and polymeric DNA, the properties of the oligodeoxynucleotide (ODN) complexes with cationic lipid and peptide carriers, in particular, their stability upon electrolyte and temperature variations, can be expected to differ significantly from the properties of the respective complexes with high molecular weight DNA [1]. Here we used X-ray diffraction, fluorescence resonance energy transfer, dynamic light scattering and zeta-potential measurements to investigate how ODNs, including an antisense ODN, known to suppress the synthesis of the anti-apoptotic BCL2 protein family in lymphoma cells, interact with cationic phospholipids and with a native polycationic peptide, protamine, which has recently been beneficially used in cationic carrier formulations [2,3].

2. RESULTS

2.1. Nanocarrier stability and DNA release in salt solutions

As evidenced by X-ray diffraction and FRET, 20-25-mer ODNs form stable lamellar lipoplexes with cationic phospholipids in water and in PBS. However, increasing the NaCl concentration to ≥ 0.3 M results in ODN release from the lipoplexes manifested as a gradual increase of the fluorescence of free DNA with increase of the salt concentration (Figs. 1 and 2).

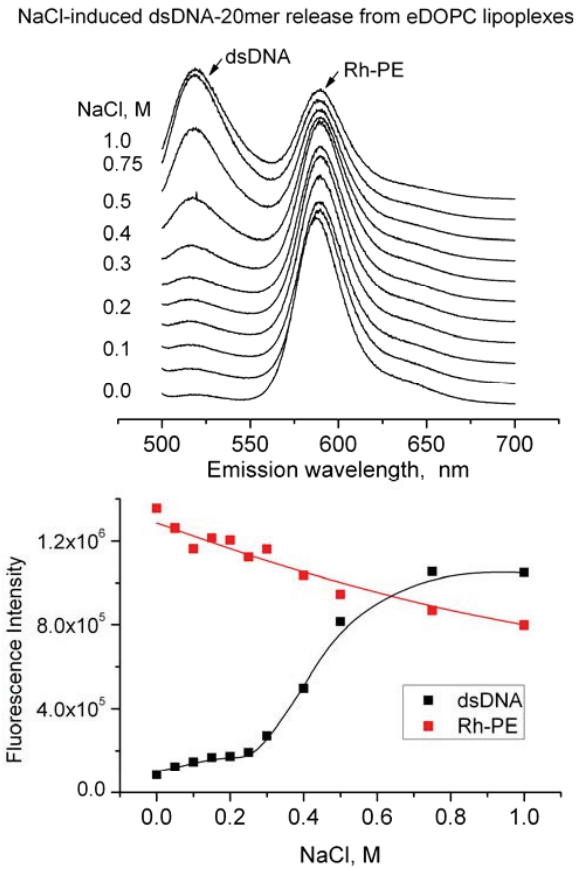

Fig. 1. Release of a fluorescent double-stranded DNA 20-mer from cationic phospholipid lipoplexes following the increase of salt concentration. DNA release can be also caused by addition of anionic lipid vesicles to the lipoplex suspension (data not shown).

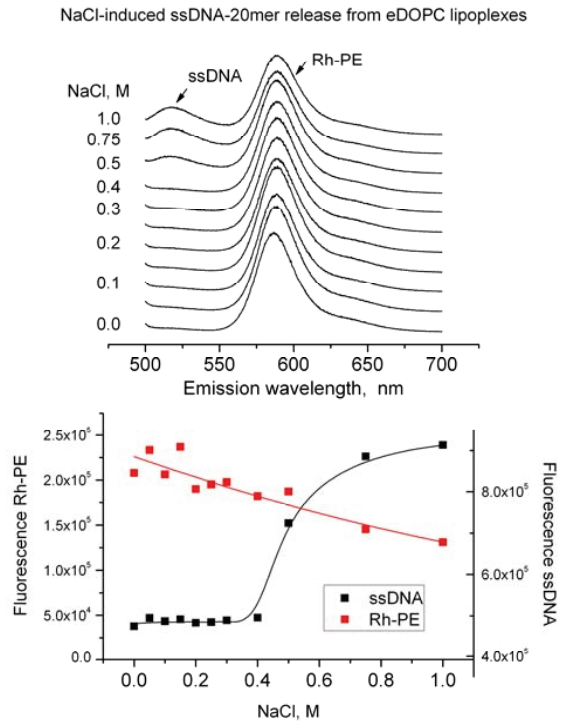

Fig. 2. Release of a fluorescent single-stranded DNA 20-mer from cationic phospholipid lipoplexes upon increase of the NaCl concentration.

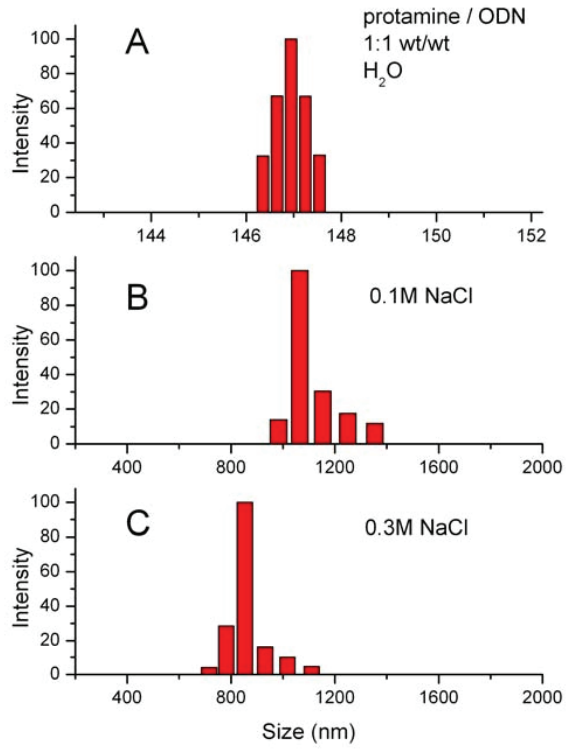

Fig. 3. Size distributions of antisense ODN/protamine nanoparticles in NaCl solutions

When mixed with protamine, all 4 kinds of DNA used in the present work (single- and double-stranded ODN, double-stranded herring sperm DNA and high molecular weight polyadenosine) spontaneously formed stable nanoparticles with remarkably narrow size distributions (polydispersity <0.1) and mean sizes of typically ~150 nm or less (Fig. 3A). The nanoparticle sizes strongly increased in NaCl solutions and in PBS (Figs. 3B, 3C and 4). Noteworthy, the size increase in NaCl solutions was not accompanied by increase of polydispersity and broadening of the size distributions (Fig. 4, bottom panel). At salt concentrations above 0.5 M protamine/ODN particles did not form,

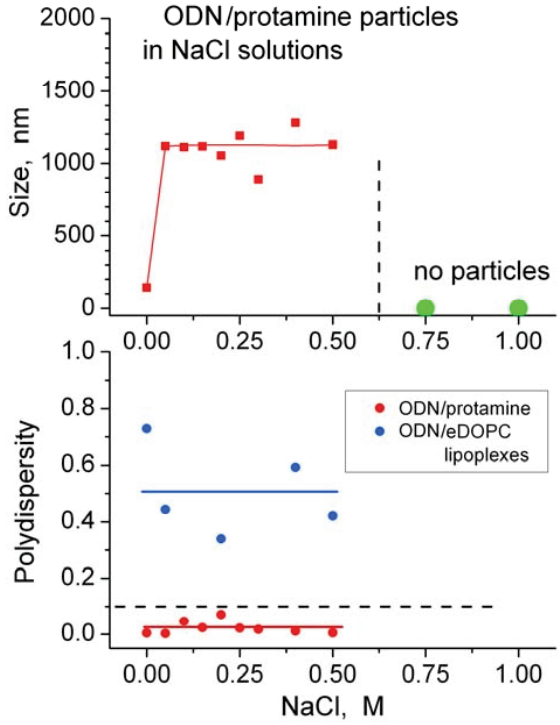

Fig. 4. Stability and size of DNA/protamine nanoparticles as a function of the NaCl concentration (top panel). The polydispersity of DNA/protamine nanoparticles is much smaller than the polydispersity of DNA/cationic lipid lipoplexes in NaCl solutions (bottom panel)

2.2. Structure of the ODN lipoplexes

According to the X-ray diffraction results, all three studied oligonucleotides (an antisense 19-mer ODN, a random 25-mer ODN and a polyadenosine 25-mer) form stable lipoplexes with cationic phospholipid derivatives. These lipoplexes are represented by tightly packed lamellar phases, which have lamellar spacings slightly smaller, by ~0.2 nm, than those of lipoplexes obtained with herring sperm DNA. However, in contrast to lipoplexes with high molecular weight DNAs, no specific DNA reflections were detectable in the diffraction patterns of the oligonucleotide

lipoplexes. This appears to indicate disordered oligonucleotide arrangements between the lipid bilayers. The ODN lipoplexes were stable and did not exhibit melting transitions upon heating up to at least 80°C. Noteworthy, the well-ordered lipoplexes made with high-molecular weight (~3 MD) polyadenosine exhibited a cooperative polyadenosine melting transition taking place at ~50°C, in very good accord with published data on the melting of free polyadenosine.

2.3. Size distributions as a function of the cationic/anionic charge ratio

The ODN lipoplexes typically have highly polydisperse size distributions with average sizes exceeding significantly the sizes of sonicated pure cationic lipid liposomes (Fig. 5).

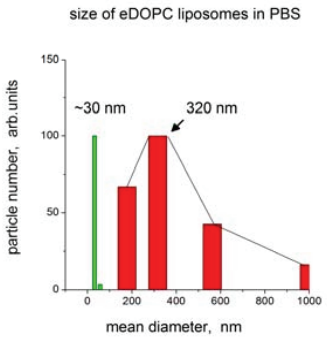

Fig. 5. Size of sonicated cationic phospholipid liposomes in the absence (green) and in the presence (red) of an antisense ODN.

The size distributions of ODN and high molecular weight DNA lipoplexes depend strongly on the anionic/cationic charge ratio. Both systems display a well expressed maximum in the isoelectric point of the particles, coinciding with the change of the sign of the particle zeta-potential (Fig. 6).

The behavior shown in Fig. 6 is in strong contrast with the behavior of the DNA/protamine nanoparticles, which do not display a maximum at their isoelectric point. The particle sizes of the latter were not affected by the type of DNA and by variation of the DNA/protamine mass ratio in the range from 1:4 to 4:1 as is illustrated by Fig. 7, top and bottom panels, although this range also includes a change of the zeta-potential sign (Fig. 7).

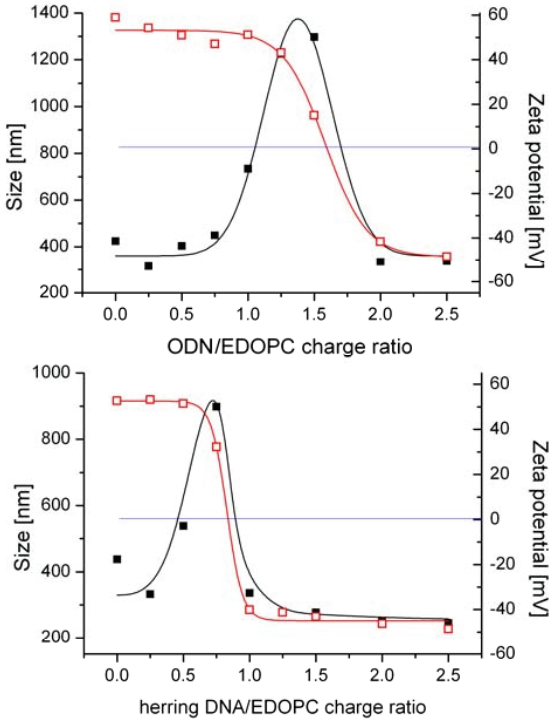

Fig. 6. Size (black line) and zeta-potential (red line) of DNA/cationic phospholipid lipoplexes as a function of the anionic/cationic charge ratio.

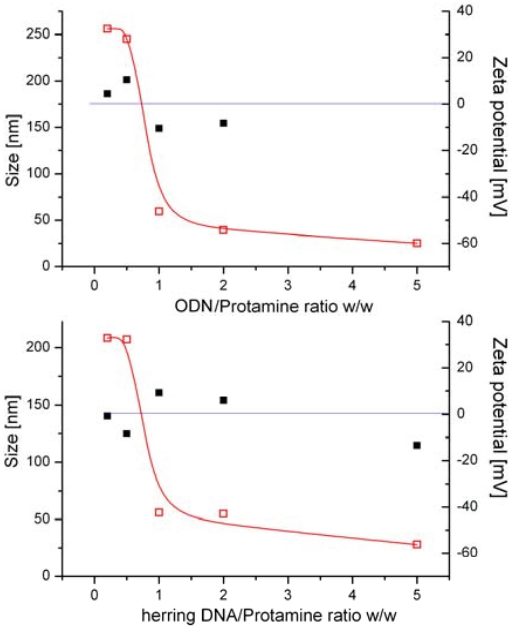

Fig. 7. Size (black line) and zeta-potential (red line) of DNA/protamine nanoparticles as a function of the anionic/cationic charge ratio.

2.4. ODN/protamine nanoparticle stability with time

The ODN/protamine nanoparticles were found to form rapidly and to reach a stable size typically within several minutes after mixing of the two components. However, in the range of the positive zeta potentials the particles were found to gradually increase in size upon storage at 4°C (Fig. 8) from ~150 nm to 250-300 nm. Remarkably, no size increase was taking place for particles with negative zeta potentials.

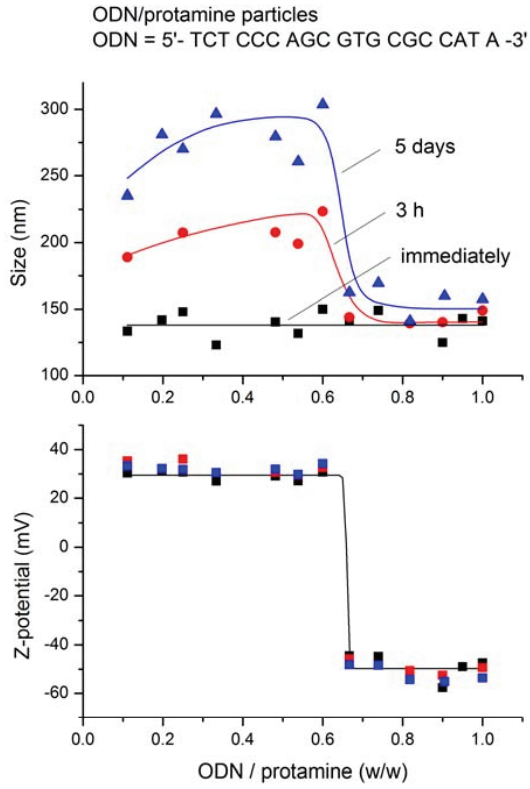

Fig. 8. Upon storage, DNA/protamine nanoparticles with positive zeta-potentials increase in size, while the sizes of particles with negative zeta-potentials remain unchanged. Noteworthy, the zeta-potential assumes two discrete, constant values of about +30 and -50 mV, which do not change with variation of the particle composition.

3. CONCLUSION

The present study demonstrated the formation of oligonucleotide/cationic carrier complexes, which are stable at physiological conditions. ODNs form stable complexes with protamine and cationic phospholipids at salt concentrations of up to ~0.5 M NaCl and ~0.3 M NaCl, respectively. The size and stability of the two types of complexes characterize them as promising oligonucleotide vectors.

4. EXPERIMENTAL

Materials: Protamine sulfate salt from salmon, Grade X, histone-free, was from Sigma. An antisense ODN, 5'- TCT CCC AGC GTG CGC CAT A -3', (A-DNA) and a random ODN 25mer (R-DNA) of 55-60 % yield were synthesized in house. Their desalting by FPLC, Sephadex G-25 column, did not have noticeable effect on the properties of the ODN/protamine nanoparticles. Herring sperm DNA from Invitrogen and polyadenylic K^+-salt from Sigma were used as received.

Solutions of DNA and protamine (0.1 and 1 mg/ml) were made in H_2O and in PBS. DNA/protamine particles were formed by mixing appropriate volumes of DNA and protamine solutions. Theirs stability was tested for NaCl concentrations up to 1 M ODN/cationic lipid lipoplexes

X-ray diffraction was used to determine the structure of ODN/cationic lipid lipoplexes. The measurements were carried out at the APS, Argonne National Lab, as described previously [4].

Fluorescence resonance energy transfer (FRET) was measured between fluorescein-labeled DNA and Rhodamine PE lipid label using an AlphaScan fluorometer (Photon Technology International).

Dynamic Light Scattering. The particle size distributions were determined by dynamic light scattering using a BI-200SM goniometer and BI-9000 digital correlator (Brookhaven Instruments, Brookhaven, NY).

Zeta potential. Zeta potentials were determined with a Malvern Zetasizer Nano instrument.

5. REFERENCES

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