KINETICS OF PLASMID DNA AND CATIONIC LIPOSOIME COMPLEXATION THROUGH IN LINE MICROCHANNEL SAXS MEASUREMENTS

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ABSTRACT
In this work we report the structural liposome modifications during DNA complexation using Small Angle X-ray Scattering (SAXS) technique. We obtained in situ complexation using an in line microchannel SAXS sample holder coupled to a fed-batch reactor and thus we were able to determine the electronic density profile and other liposome parameters in the presence of DNA.

KEYWORDS: Liposome, Kinetics, DNA, Complexation, SAXS

INTRODUCTION
Gene therapy is an exciting research area that allows the treatment of different diseases. Basically, an engineered DNA that codes a protein is the therapeutic drug that has to be delivered to the cell nucleus. After that, the DNA transfection process allows the protein production using the cell machinery. However, the efficient delivery needs DNA protection against nucleases and interstitial fluids. In this context, the use of cationic liposome/DNA complexes is a promising strategy for non-viral gene therapy. Liposomes are lipid systems that self-aggregate in bilayers and the use of cationic lipids allows the electrostatic complexation with DNA. Cationic liposomes protect the DNA from extracellular matrix and shuttle it inside the cell [1]. Depending on the lipid composition, molar charge ratio between positive (from cationic lipids) and negative charges (from the DNA phosphate group) \((R_{+/-})\), complexes with different characteristics can be obtained. Although cationic liposomes/DNA complexes have been extensively studied, the kinetic of complexation still demands efforts.

In this work, we used SAXS technique to study the complexation kinetics between cationic liposomes and plasmid DNA and evaluate the liposome structural modifications in the presence of DNA.

EXPERIMENTAL
Liposomes were composed of Egg phosphatidylcholine (EPC), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) and 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) (50/25/25% molar) (Lipoid) [2]. Liposomes were prepared by thin film method using a rotary evaporator in a 650 mmHg vacuum for 1 h. The dried lipid film was hydrated with PBS buffer to a lipid final concentration of 16 mM. The prepared liposomes were extruded through polycarbonate membranes (100 nm nominal diameter) 15 times under a nitrogen pressure of 12 kgf/cm².

We used as plasmidial DNA vector model a modified version of pVAX1-GFP with luciferase as reporter gene [3]. The plasmid DNA was amplified in Escherichia coli bacteria and purified using PureLink™ HiPure Plasmid DNA Purification Kit-Maxiprep K2100-07. The concentration of DNA and its purity was determined using Nanodrop UV spectrophotometer ND-1000. The A260 nm/A280 nm ratio of 1.8 was used as the criteria for purity.

The plasmid DNA and cationic liposome complexation was performed in a fed-batch reactor under magnetic stirring in ice bath as illustrated in Figure 1. The DNA solution (500 µg/mL) was continuously injected through a syringe pump (26.7 µg/min) in the liposomal dispersion. The molar ratio between the charges of the cationic lipid and the DNA was gradually decreasing from “empty” liposome \((R_{+/-}=∞)\) to \(R_{+/-}=5\). Using a peristaltic pump, a recycle line continuously pumped the bulk complex from the reactor to the sample holder. The SAXS sample holder was constituted of a microchannel with diameter of 600 µm to get access to the compartment between two mica windows where the X ray beam could cross through.

The energy of the incident beam was 8 keV \((λ=1.55 Å)\) and detector distance was \(≈1600 mm\). The scattering intensity was acquired by a Pilatus detector 300k (Dectris) and a typical range in \(q\) was from 0.007 Å\(^{-1}\) to 0.224 Å\(^{-1}\).

The average hydrodynamic diameter and size distribution and zeta potential were measured using Malvern, Zetasizer Nano ZS.
RESULTS AND DISCUSSION

We used SAXS to study the influence of DNA on the structure of the liposomes. Figure 2 shows the scattering intensity curves during the kinetics of complexation with molar charge ratio (R_{+/-}) ranging from “empty” liposomes (R_{+/-}→∞) to R_{+/-} = 5 (Increasing the amount of DNA, decreases R_{+/-}). The electronic density profiles were determined through the scattering curve fitting using a decoupling method which considered both, form and structure factor for the scattering centers. Figure 3 shows the electronic density modification of the liposome bilayer during the complexation. Table 1 shows the results on interlamellar space (L), number (N) of bilayers and the Caille parameter (η) for three selected conditions. The results indicate an increase of the interlamellar space and number of lamellae in the multi-lamellar liposome form in the presence of DNA. The Caille parameter is slightly bigger after the complexation indicating that the lamellae become more fluid. These results are in agreement with prior reported studies [4].

Table 1. Interlamellar space (L), number of bilayers (N) and the Caille parameter (η) for three conditions obtained by SAXS.

<table>
<thead>
<tr>
<th>Condition</th>
<th>L (Å)</th>
<th>η</th>
<th>N</th>
</tr>
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<tbody>
<tr>
<td>“Empty” cationic liposome</td>
<td>77.1±0.2</td>
<td>0.12±0.01</td>
<td>2.15±0.02</td>
</tr>
<tr>
<td>Cationic Liposome/DNA (R_{+/-} = 9)</td>
<td>75.9±0.1</td>
<td>0.15±0.02</td>
<td>2.33±0.02</td>
</tr>
<tr>
<td>Cationic Liposome/DNA (R_{+/-} = 6)</td>
<td>75.6±0.1</td>
<td>0.16±0.02</td>
<td>2.28±0.02</td>
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The structural changes observed during the complexation reaction can be explained by previously reported study [3], in which three main steps can occur. In the first step (millisecond time scale), DNA binds to the lipid bilayer surface, generating compressive stresses. The second step (order of seconds), there is an intermediate state with the formation of a locally cylinder-like structure around the DNA axis. This intermediate state is very unstable, and in order of minutes, there is a disrupting of the liposomes lamellae for its number. In the last step, the accommodation of these new surface occurs, generating additional vesicles.

The hydrodynamic diameter were evaluated in three different conditions (“empty” liposomes, cationic liposome/DNA complex at R_{+/-} = 10 and 5) (Figure 4). We can observe an increase in the mean diameter at higher R_{+/-}.

CONCLUSION

In this work we reported the structural liposome modifications during DNA complexation using small angle X ray scattering (SAXS) technique. It was possible to identify the increase in the number of lamellae and decrease of the interlamellar space of the liposome after addition of DNA. The bilayer fluidity and electronic density profile were characterized. These differences can reflect in singular in vitro and in vivo effects.
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REFERENCES

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