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REPORT

Beamline:	**Dates of the experiment:**
ID02	21/04/2023 to 24/04/2023
Proposal ID:	**Local contact:**
SC-5377	Gouranga Manna

Names and affiliations of applicants:

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Name: Effect of charge ratio and polycation structure on early stage kinetics of nucleic acid-based complex formation studied through stopped-flow SAXS

Abstract: The study of polyelectrolyte complexes (PECs) for applications in nucleic acid-based therapies is currently attracting great interest due to the emergence of mRNA vaccines. While many complexes have been examined in gene transfection assays, only few studies have investigated the ultrafast kinetics of nucleic acid complexation. Recently, we used the stopped-flow (SF) light scattering (LS) technique to study the early-stage complexation kinetics of a model system composed of calf-thymus (CT) DNA and chitosan. Time-Resolved Small Angle X-ray Scattering on ID02 is the best suited approach to study the complexation mechanism. Our goal is to determine the size and morphology of complexes formed at the early-stage kinetics and to explain the effect of charge ratio and polycation structure on complexation mechanism. This report describes first results within a more ambitious project that aims at elucidating the different steps of the disassembly of nucleic acid-based complexes in physiological conditions.

Background:

The initial steps of PECs formation taking place on a millisecond time scale, have been scarcely studied and almost none with DNA. We firstly have started determining the early-stage kinetics of complex formation CT-DNA/chitosan that has been widely used in our group. Firstly, multiangle light scattering was used to study the morphological organization of the complexes, which was found to be mostly dependent on chitosan molecular weight (MW). A globular morphology of complexes formed with high MW chitosan samples appear to be in good agreement with a core–shell structure often proposed for polycation-DNA complexes at R > 1. PECs formation performed by SF mixing with a LS detection showed two distinct kinetic processes. The first one was detected at ~1 s for R < 1 (*Figure 1a*) and few hundred ms for R ≥ 1 (*Figure 1b*) and can be ascribed to primary complexes formation. The second process, observed at ~ 12 s, may correspond to the formation of secondary complexes based on a dense core surrounded by shell of excess polyelectrolyte. The first observed process was found to be dependent on chitosan MW and R, contrary to the second one, which was almost constant for all the samples.

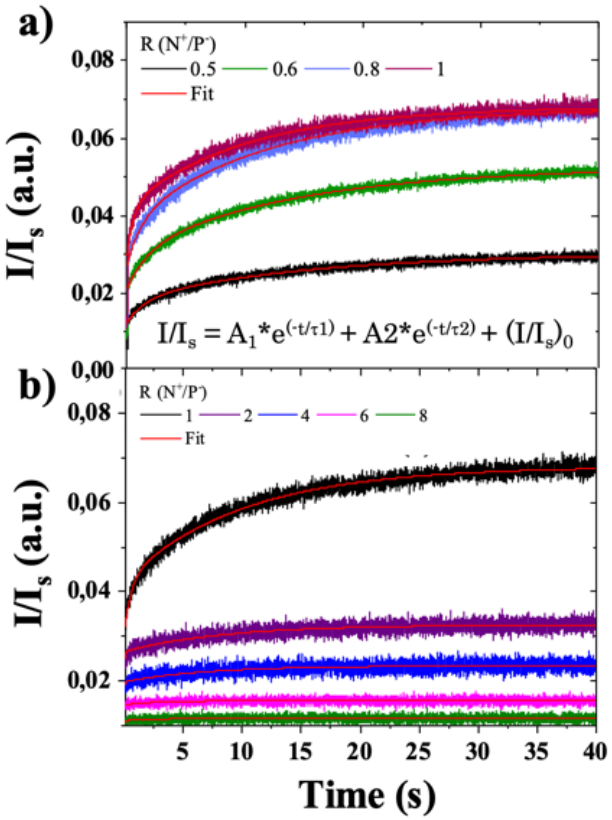

Figure 1.- DNA/chitosan complexation kinetics followed by SF-LS. for a) R = 0.5, 0.6, 0.8 and 1; b) R = 1, 2, 4, 6 and 8. Scattered intensity was normalized with the scattered intensity of the solvent. The continuous lines represent the resulting fits obtained by using a double exponential expression.

Experimental results:

The objectives of the experiments were to follow the complexation mechanism of chitosan/calf-thymus DNA complexes through Small-Angle X-ray Scattering, to access to information about resultant morphologies formed at the early-stage kinetics, and to explain the effect of charge ratio and polycation structure on the complexation mechanism.

We selected three chitosan samples (ChitoClear®, Northern coldwater shrimp, Primex, Iceland) to study the effect of the MW on complexation kinetics: Chitosan #1 (510 kg/mol, DA=0.30), Chitosan #2 (350 kg/mol, DA=0.28) and Chitosan # 3 (210 kg/mol, DA=0.29), where DA is the degree of acetylation and is very close in all the samples. Calf-thymus DNA (CT-DNA, with a MW= 6,6 x 10^5 g/mol) was used as a model nucleic acid chain. The effect of charge ratio (R=$[N^+]/[P^-]$, where $[N^+]$ corresponds to the concentration of chitosan protonated amino groups and $[P^-]$ corresponds to the concentration of DNA

negatively charged phosphate groups) was studied by performing stopped-flow measurements at: R= 0.5, 0.6, 0.8, 1, 2, 4, 6, and 8. Static SAXS experiments on complexes final state prepared by one-shot addition outside the beam were also performed for comparison.

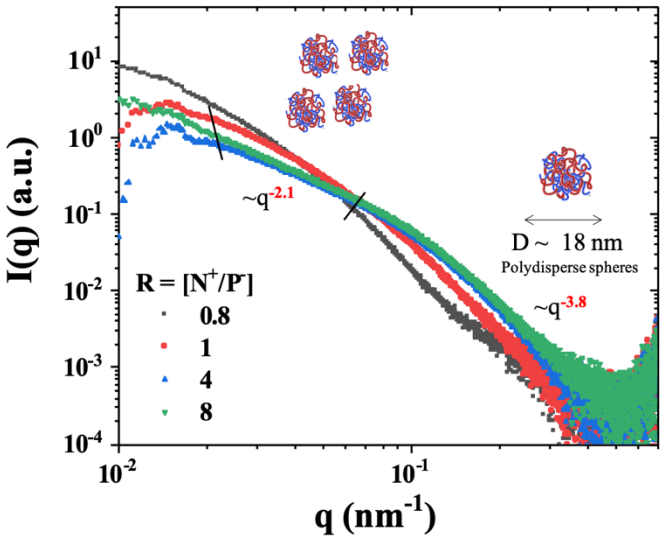

Figure 2.- SAXS intensity I(q) as a function of the wave vector q for CT-DNA/chitosan complexes prepared at different charge ratios: R= 0.8, 1, 4 and 8.

Figure 2 presents, as an example, background-subtracted SAXS data for CT-DNA/chitosan #1 complexes prepared at four different charge ratios (R= 0.8, 1, 4 and 8). The pattern could be divided in three scattering domains. For CT-DNA/chitosan complexes prepared at R≥1, at high q values, a Porod-like regime was found, with scaling behavior around $q^{-3.8}$, which is compatible with the morphology of well-defined, dense particles. At intermediate q values, the behavior could be modeled by using a polydisperse sticky hard sphere fit. Finally, primary particles observed at high q may probably aggregate, giving rise to a $q^{-2.1}$ dependence at medium-low q, suggesting a diffusion-controlled aggregation mechanism. In the Porod representation and assuming a spherical morphology, analysis allowed obtaining information about the sphere radius, which was found to be 8.7 ± 4.4 nm for a R= 4 CT-DNA/chitosan complex, consistent with the size obtained from analysis of Atomic Force Microscopy (AFM) images for the small particles (*Figure 3*). For all charge ratios and the three chitosan MW, the complexes formation led to very aggregated systems, revealed by SAXS through the missing clear-cut Guinier behavior at small q, in good agreement with AFM observations.

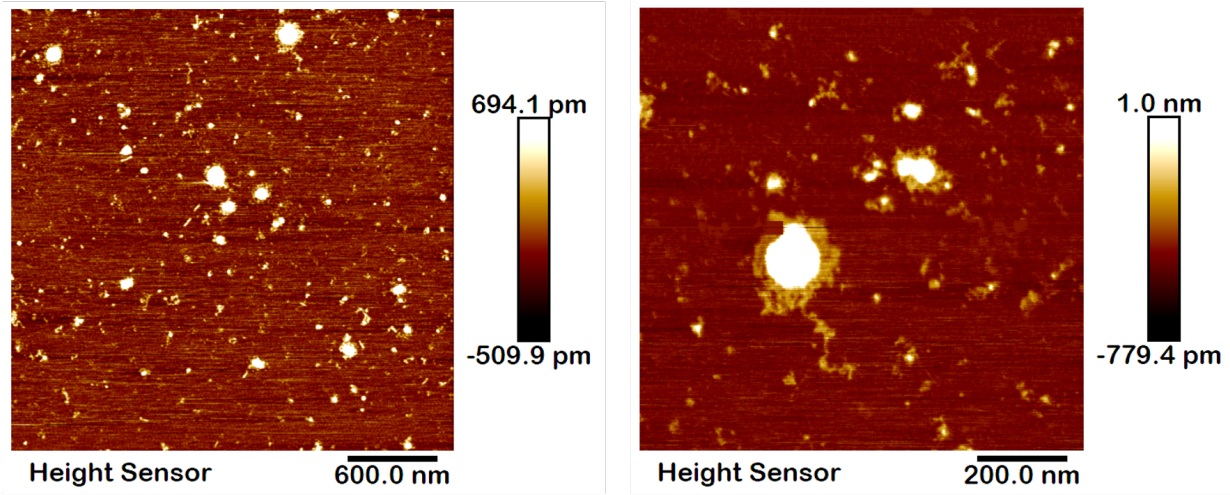

Figure 3.- AFM images for CT-DNA/complexes prepared at R= 4.

Figure 4 shows the typical time-resolved SAXS profiles for complex formation between chitosan #1 and CT-DNA at the initial and final mixture time for R=0.6 (a) and R= 6 (b). As *t* elapsed, the scattering intensity in the intermediate *q* region increased for both charge ratio, particularly more for R= 0.6, *i.e.* corresponding to less condensed complexes having an excess of DNA. From preliminary analysis of SAXS profiles for complex formation at R=6, prepared with chitosan #1, the initial complex was found to be smaller at the first milliseconds, however, it seems that the spherical structures are already formed from the very beginning of the SAXS acquisition. A different pattern evolution can be observed for complexes prepared at R ≤1, which will lead to a slower evolution of complex organization with time.

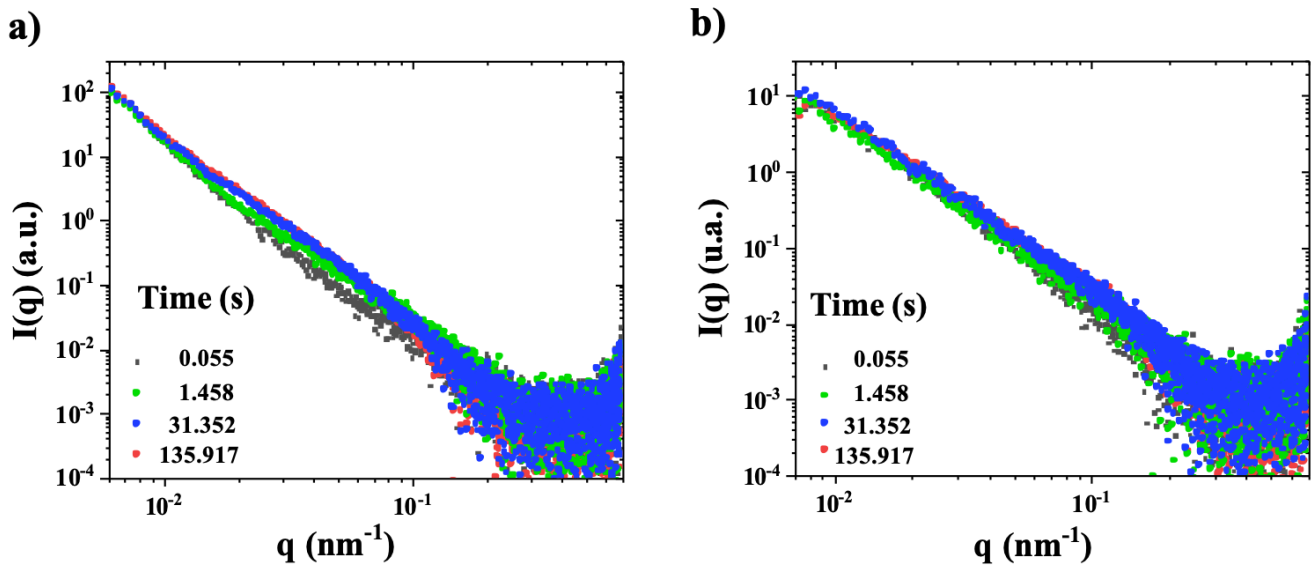

Figure 4.- SAXS intensity I(q) as a function of the wave vector *q* for CT-DNA/chitosan complexes, prepared at R= 0.6 (a) and R= 6 (b) taken during the complex formation at the initial and final measurement time.

Ongoing analysis:

SAXS profiles obtained during complex formation for all CT-DNA/chitosan complexes, prepared with the three different MW Chitosan samples at various charge ratios, at different times from stopped-flow measurements, are now under analysis and fitted with the sticky sphere model. Data fitting will provide small spherical particles size evolution as a function of R and chitosan MW during complex formation. The evolution of the slopes at the high and low *q* regions will be also determined. The corresponding publication will be written after the complete anlysis of the data.