Probing DNA assembly into nanoparticles with short DNA

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ABSTRACT

DNA is an anionic polyelectrolyte, which occupies a large volume in salt free solution due to the coulomb repulsion between the charged groups. In the presence of high valence cations, DNA condenses into nanoparticles. DNA nanoparticles have generated a lot of interest as a preferred vehicle for delivering therapeutic DNA in gene therapy. The efficiency of gene delivery is determined by stability and compactness of the particles. However not much is known about the organization of DNA within the particles. The large polymer cations condense DNA rapidly, with no distinct intermediate stages that give insight into the arrangement of DNA within the nanoparticle. In our work, we form nanoparticles with short DNA strands to slow down the condensation process. The polymer cation is polyethyleneimine with grafted sugar moieties. Distinct intermediate stages are observed with Atomic Force Microscopy. The assembly occurs via the formation of fiber condensates, which appear to be the unit of DNA condensation. Nanoparticles form by compaction of interweaving networks of fiber condensates.

INTRODUCTION

DNA is a highly negatively charged polymer. It is semirigid (persistence length of 50 nm) and self-repelling, occupying an expanded volume. However in the presence of polycations (3 positive charges or higher), DNA can be condensed to less than 1000 times its free volume; allowing several micrometers of DNA be packed into viruses and bacteria of nanometer size. DNA condensation with polycations has generated a lot of interest both from a basic sciences viewpoint to understand DNA packaging in viruses, but also for packaging DNA into nanoparticles for delivery into cells.

The human genome project and the prospect of combating diseases by delivery of therapeutic genes has fuelled a lot of research into using DNA nanoparticles as gene delivery agents [1]. Commonly used cationic polymers like polyethyleneimine condense DNA into stable, compact particles [1, 2]. The net positive charge on the nanoparticles allows attractive interaction with the negatively charged cell surface [2]. The nanoparticle size is on the order of virus particles, and is taken up within the cell by the preferred endocytosis machinery. The buffering action of polyethyleneimine protects the DNA from the acidic environment of the endocytosis vesicles.

It is increasingly appreciated that small changes in the DNA organization within the nanoparticles have large influences on the biological activity of the particles [3]. Therefore a systematic study of DNA organization within nanoparticles and its correlation with biological activity is warranted. However, for DNA lengths typically studied (> 10 kb) and for condensation with polymer cations, nanoparticles form rapidly at around charge neutralization ratios [4, 5]. Due to the rapid condensation, distinct intermediate stages cannot be obtained, which would allow visualization of the DNA assembling into nanoparticles. Intermediate

structures have been reported in literature, and they typically involve fibrous strands surrounding a globular region [6]. It is not clear if these are intermediate, disrupted, or badly formed nanoparticles. Moreover the DNA arrangement in the globular core is not known.

Several researchers have reported that the average nanoparticle size is relatively independent for a wide range of DNA lengths [7]. This suggests that the interactions leading to nanoparticle formation are conserved across a wide range of DNA lengths. We hypothesize that since multiple molecules of a relatively short DNA will be involved in the formation of an average nanoparticle, the process of particle formation will be slowed down by the kinetics of the DNA-DNA approach. As particle formation becomes slower, we expect to see distinct transition stages, which would provide insight into the DNA organization within these objects.

MATERIALS

Nanoparticles were formed using the polymer cation polyethylenimine (Sigma Chemicals). A 22 kDa linear form (MW 13.6 mM) was used which has about 500 secondary amine groups separated by ethylene groups (Fig. 1). The polymer was also additionally grafted with 3 % grafted mannobiose [5]. We refer to it as PEIm.

Figure 1. Repeating unit of linear polyethylene imine

We used 1kb linear DNA to probe the transition stages involved in nanoparticle formation. At 1 kb, the DNA length is still many times longer than the persistence length, and we do not expect entropic effects to alter the interactions involved in the condensation pathway. For comparison, we also formed nanoparticles from 12 kb plasmid DNA. Nanoparticles were prepared by adding DNA to a PEIm solution in NaCl, to obtain a final concentration of 1ng/μl DNA, 1.36 mM PEIm and 50 μM NaCl. For this formulation N:P > 4. N and P refer to the protonated PEIm nitrogen and the DNA phosphate anion, respectively. The condensate solution was incubated for 12 hours to form nanoparticles. The pathway to nanoparticle formation was determined by sampling the incubate solution at intermediate times of 0.5 hour and 1 hour.

The condensates were imaged using Atomic Force Microscopy (AFM). 2 μl of the condensate solution was placed on freshly cleaved mica and allowed to air dry. Veeco Multimode system was used for imaging in tapping mode in air. Silicon Cantilevers (OMCL, ~300kHz, 42N/m) were used. In addition to AFM height images, we made AFM phase images. These were obtained by tracking the phase of the cantilever vibration and resulted in a clearer 3D visualization of the particle surface. Particle analysis was performed with NIH ImageJ software. Particles of diameter < 5 nm were not used in the image analysis.

EXPERIMENT

Fig. 2 compares the nanoparticles formed from 12 kb and 1 kb DNA after 12 hours incubation. By 12 hours, the nanoparticles from 12 kb plasmid DNA (Fig. 2a) are completely formed (Fig. 2b). In the AFM phase images, the particle surface is well formed, spherical and smooth (inset). On the other hand, at 12 hours incubation, the nanoparticles of the 1 kB DNA (Fig. 2c) appear less spherical, and less well-formed (Fig. 2d). In the phase image, the particle surface appears corrugated with rich patterns, revealing the DNA arrangement within.

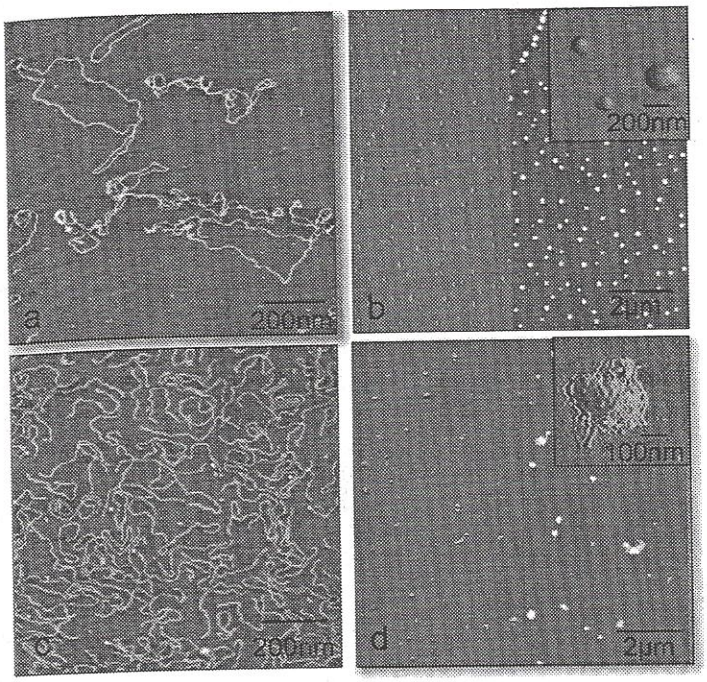

Figure 2. (a) 12 kb plasmid DNA. The scan area is 1 μm X 1 μm. (b) Nanoparticles formed by condensation of 12 kb pDNA with PEIm. The scan area is 10 μm X 10 μm. The left half is the AFM phase image, and the right half is the AFM height image. Together they show that the nanoparticles have a spherical shape with a smooth surface (left). In the enlarged view of the nanoparticle surface (inset), it appears that the DNA is tightly packed within the particles, with no visual clues about its organization. (c) 1 kb linear DNA. The scan area is 1 μm X 1 μm. (d) AFM image of nanoparticles formed by the condensation of 1 kb DNA with PEIm. The scan area is 10 μm X 10 μm. The phase image is shown on the left and the height image is shown on the right. The nanoparticles are less spherical, and less tightly distributed in size. The phase image of the particle surface (inset) reveals intricate patterns of a fiber meshwork.

The diameter of the nanoparticles was determined from their volume on the imaging surface. The diameters of both the 12 kb and 1 kb nanoparticles are distributed about a mean value of 50 nm (Fig. 3). However the distribution of the 1 kb particles is wider, which is expected since there are more integral copies of DNA coming together to form the same particle. As expected, the 1 kb nanoparticles formed relatively slowly and distinct transition stages could be visualized at intermediate times (Fig. 4). Several short, thick fibrils were seen at early times (Fig. 4b). They appear to be forming from DNA (arrows) condensing along its length. These fibril condensates also appear to be 'sticky'. They associate in parallel and intertwining manner to form longer fibers, simple branched structures, and inter-weaving networks. The inset in Fig. 4b shows the corresponding phase image of one such network. The taller 'core' region observed in the height image actually bears the same inter-woven fibril structure as the rest of the network, but only in a significantly more compact or close-packed state. This suggests that the compaction of inter woven networks of fiber condensates gives rise to DNA nanoparticles. This is evident in the intermediate-stage nanoparticles shown in Fig. 4c (1 hour incubation). The nanoparticles have a compact core surrounded by fiber condensate meshwork, which is still in early stages of

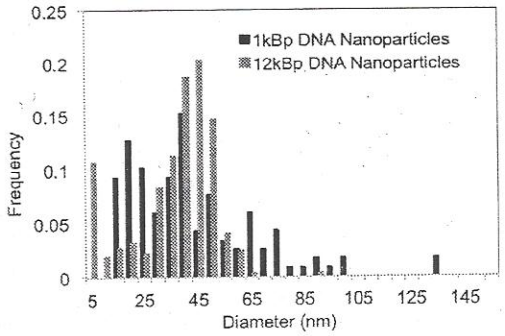

Figure 3. Histogram of nanoparticle diameter calculated from the particle volumes. The 12 kb (grey) and 1 kb (black) DNA are distributed normally about a peak diameter value of 42.5 nm. The 1 kb DNA nanoparticles are more widely distributed in diameter.

compaction. At 12 hours, most of the network compaction appears complete (Fig. 4d). The phase image of a particle surface (inset) shows that the condensed core is continuous with surrounding network and has a similar interwoven structure. Interestingly, some particles have an aggregate-like shape due to the presence of multiple compaction cores in the initial network.

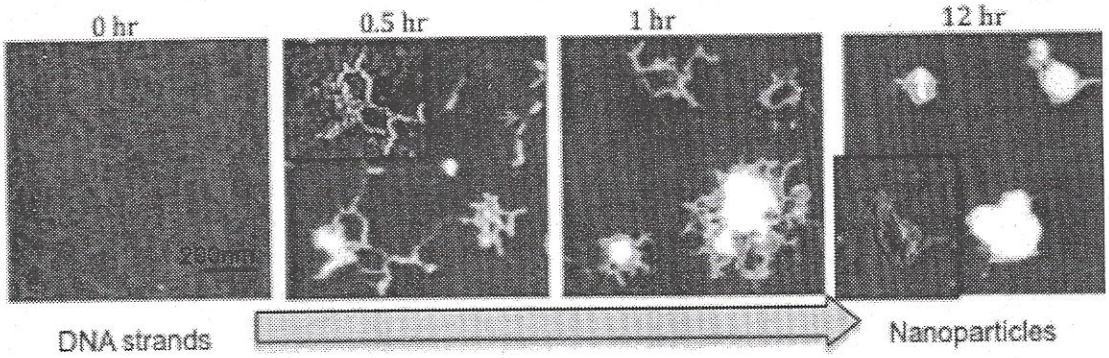

Figure 4. Time profile of the formation of nanoparticles from 1kB DNA. (a) 1kb DNA at 0 hours (b) At 0.5 hours, one can see DNA (arrow) condenses along its length into fiber or rod-like structures. These fiber condensates appear to associate with each other into interwoven networks. The phase image of one such network (inset) shows that its taller core is also composed of interweaving networks of fiber condensates, but in a closely packed state. (c) At 1 hour, nanoparticles appear form by progressive compaction of the fiber condensation networks around a localized core. (d) At 12 hours, the nanoparticles are nearly formed. The condensation core encompasses the original network, which appears to be in its final stages of compaction. The phase image shows the structure of a condensed core (inset). It still retains the structure of the fiber condensate network it originated from. Each image area is 1 μm X 1 μm.

The fiber-condensates appear to be the unit of DNA organization in the nanoparticles. While they appear to be forming from DNA condensing along its length, it is not clear if they are composed of single or multiple DNA. We did a volume histogram of the intermediate structures present in the 0.5 hour condensation incubate (Fig. 5a). A clear peak centered at 1e-6 μm^3 was obtained, which corresponds approximately to the volume of one 1 kb DNA. The next peak was about the volume of two 1 kb DNA. The length distribution of the particles constituting the one DNA volume peak shows one maximum at 55 nm length and another at 70 nm length (Fig. 5b).

The former corresponds to globules formed from full condensation of a 1 kb DNA, and the latter corresponds to the fiber condensates seen earlier. The results show that fiber condensates with the volume of 1 kb DNA and approximately one-quarter of its extended length, is a consistent feature in the pathway of nanoparticle formation.

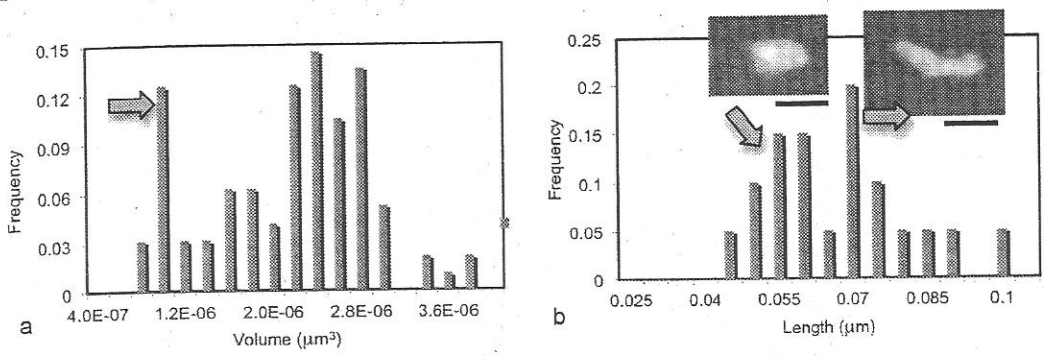

Figure 5. (a) Volume analysis of the intermediate 1 kb nanoparticle structures. There is a prominent peak which corresponds to the volume of one 1 kb DNA. (b) Length analysis of structures contributing to the 1 kb volume peak (arrow). A sharp peak is seen at 70 nm and a more distributed peak at 55 nm. The 70 nm peak was attributed to fibril condensates and the latter to globule-like structures (insets). A single DNA can therefore condense either into fibrils, which are about one-quarter of its length, or into globular structures. Interactions between the condensed fibrils form larger nanoparticles. The scale bar is 50 nm.

CONCLUSIONS

We are interested in the organization of DNA within nanoparticles formed by condensation induced by large polycations. Since nanoparticles from large DNA form rapidly and have a rather smooth surface, not much is known about the DNA organization within. We show that nanoparticle formation can be slowed down using short DNA, possibly due to the time taken for multiple DNA to associate to form particles of similar size.

Several intermediate stages are distinguishable in the formation of nanoparticles from 1 kb DNA. It always involves a thicker condensed form of DNA, which we refer to as 'fibril condensate'. The fibril condensates appear to associate readily, and form simple branched structures and intertwining networks. Over time, these fiber condensate networks become compact or pack tightly to form localized condensation cores. Nanoparticles form as the fiber condensate network around the core compacts progressively. Phase images of nanoparticle surface show that it retains the inter-weaving pattern of the initial fiber condensate network.

The fiber condensates appear to be the DNA condensation unit. The structure of DNA within these condensates is not precisely known. In separate studies we found that the trivalent cation, spermidine, also condenses DNA into fiber condensates of similar dimensions. Other published studies had also demonstrated the formation of rod-like fiber condensates in the condensation pathway [8]. Volume and length analysis of nanoparticle intermediates suggests that the fiber condensate form from a single DNA, and is about one-quarter of its length. They can also undergo complete compaction into globules.

There are many possible pathways for the formation of nanoparticles, and in this study we explored one of these. We use a minimalistic condensation solution that does not involve buffer.

This made it possible to image intermediate stages without washing the sample before imaging, and removing structures that do not adsorb well onto the imaging surface. For the DNA and polycation concentrations in our solution, we find that DNA condenses along its length before interacting with each other. We expect the pathway to be different in more concentrated DNA solutions, where the inter-DNA interaction can occur more rapidly than the intra-DNA condensation. Moreover, while rods and fiber condensates are typically observed in DNA condensation solutions and appear to be independent of the polycation used; it is not known if the pattern of fiber condensate association is specific to a given polycation. The effect of the different possible condensation pathways on the biological activity of the nanoparticle is an important area to explore.

To summarize, we investigated the organization of 1 kb DNA within nanoparticles formed by condensation with the polymer cation PEIm. Our main findings are:

1. Condensation initiates by DNA folding along its length into thick fiber condensates.
2. Nanoparticles form by the compaction of interwoven of associating fiber condensates.

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