Nucleation of DNA Condensation by Static Loops: Formation of DNA Toroids with Reduced Dimensions

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Controlling DNA condensation is presently of interest for the development of nonviral approaches to gene therapy.[1] Toward this end, a number of studies have investigated the relationship between the structure of novel DNA condensing agents and the morphology of their associated DNA condensates.[2] Additionally, a wide range of multivalent cations have been studied which condense DNA into toroidal structures.[3] In contrast, the effect of DNA structure (e.g. bends) prior to condensation has received far less attention.[4] Here we report that the introduction of localized static curvature into an otherwise linear duplex DNA polymer can have a profound effect on the size of toroidal condensates formed when the entire polymer is condensed by multivalent cations.

Previously, we presented a model for DNA toroid formation in which the initial step is the spontaneous formation of a single loop along the DNA polymer.[5] This loop was proposed to act as a nucleation site for DNA condensation and to be responsible for defining the size and morphology of the toroid. These loops would form as the result of random polymer fluctuations in solution, with their size being a function of the persistence length of DNA. Such a process is supported by real-time studies of DNA condensation which indicate that toroid formation follows a nucleation-growth pathway.[6] The loop initiation model for toroid formation also suggests a mechanism by which toroid size could be altered. That is, if a static loop or multiple loops were introduced into an otherwise linear DNA polymer, these loops would provide a site for toroid nucleation temporally favored over loops formed by random polymer fluctuations. Controlling the size of these static loops would then provide a means for altering toroid dimensions.

To investigate the effect of static loops on toroid formation we examined the condensation of a DNA polymer with and without the incorporation of curved DNA. This curved DNA was produced by A-tract sequence-directed curvature. Briefly, an A-tract is a DNA sequence of four to eight consecutive adenine residues.[7] A single A-tract can produce a bend as large as 20° in the helical axis of duplex DNA,[8] and long-range static curvature is produced by multiple A-tracts if their incremental bends are in phase with the helical repeat of DNA (∼10.5 bp). We have produced a series of DNA polymers based upon a 2961 bp linearized plasmid DNA (pBluescript II SK-, Stratagene, La Jolla) which contain an insert of one, two, three, or four tandem copies, respectively, of the 173 bp sequence 5′-ATCCATCGACC-(AAAAAACGGGCAAAAAACGGC)$_7$AAAAAAGCAGTGGA-AGC-3′ (Figure 1).[9]

DNA polymers (10 μg/mL in TE buffer, pH 7.5) were condensed from solution by the addition of hexammine cobalt(III) (100 μM) and examined by transmission electron microscopy (TEM) (Figure 2).[9] Toroidal condensates produced by polymers containing two or more copies of the 173 bp A-tract insert typically measured 45 nm in outside diameter with a 10 nm diameter hole. Assuming a circular cross section and hexagonal packing between DNA helicies,[10] these toroids could contain up to three of the approximately 3 kb DNA polymers.

DNA polymers without the A-tract inserts (i.e., linear pBluescript II SK-DNA) were also examined after condensation by hexammine cobalt(III). These polymers produced toroids which measured 130 nm in outside diameter with a 40 nm diameter hole (Figure 2). The amount of DNA in these toroids could be in excess of fifty 2961 bp polymers per toroid.

The axial bend of a single A-tract (i.e. A_6) has been estimated to be 17−21°.[8a] Thus, the 15 phased A-tracts of our 173 bp insert sequence would be expected to produce an arc of 255−315° with a radius of curvature as small as 10 nm. Electron micrographs of partially condensed DNA polymers containing four tandem copies of the 173 bp A-tract sequence reveal DNA circles with a radius of curvature of approximately 12.5 nm (Figure 3), which corresponds to 230 bp per circle. This is in good agreement with the previous estimate for A-tract induced bending, and also confirms that a single copy of our 173 bp insert would be insufficient to produce a complete circle. The similar dimensions of the small toroids (Figure 2A) and the circles of partially coiled DNA (Figure 3A), as well as the apparent lack of small toroids in the condensates of DNA polymers containing only one copy of the 173 bp insert (unpublished data), support the formation of small toroids as being the result of toroid nucleation on static circles produced by the curved A-tract inserts.

DNA polymers over a wide range of lengths (i.e., <1 to 48 kb) have been shown to produce toroids of approximately 100 nm in outside diameter,[11] with the toroids formed by shorter polymers containing a correspondingly greater number of poly-

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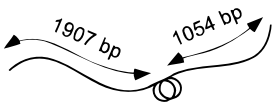

Figure 1. Schematic representation of the DNA used in this study, a 2961 bp plasmid DNA (pBluescript II SK-) with (and without) A-tract repeats introduced near the polymer center. Circles represent one, two, three, or four tandem copies, respectively, of the 173 bp sequence presented in text.

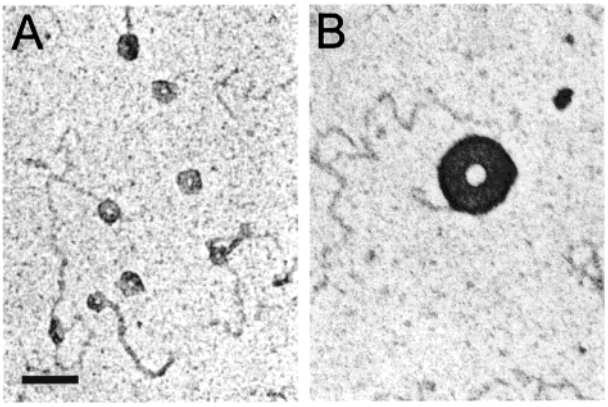

Figure 2. (A) Transmission electron micrograph of toroids produced by the condensation of the linear 2961 bp DNA polymers with four tandem copies of the 173 bp A-tract repeat inserted near the center of the original polymer. (B) Micrograph of a toroid produced by the condensation of the linear 2961 bp DNA polymer without any A-tract inserts. (Bar = 100 nm.)

mers per toroid. The dimensions of the larger toroids shown in Figures 2B and 3B are consistent with previous reports for toroids resulting from the condensation of approximately 3 kb DNA. However, polymers with two or more A-tract inserts produced smaller toroids (Figure 2A) than polymers without the inserts (Figure 2B). Thus, the nucleation of toroids on the curved DNA inserts not only resulted in the generation of toroids with DNA coiled into a much smaller average radius of curvature, but also limited the number of polymers contained in each toroid. We propose that this is because each larger toroid was spontaneously nucleated on a DNA molecule which then proceeded to incorporate many other molecules before they had successfully nucleated toroids of their own, whereas the small toroids completed nucleation faster or in a more synchronous manner. Larger toroids, similar in size to those produced in the absence of static loops, were also observed at a lower frequency (as low as ~2%) in condensates of plasmids with A-tract inserts that primarily produced small toroids (Figure 3). This indicates that the formation of large toroids is *not* blocked by the introduction of the A-tract repeats, providing additional support that smaller toroids are only kinetically favored over the larger ones.

Anomalously small toroids have previously been reported in the condensates of homogeneously curved DNA.[4c] However, our results demonstrate that *localized* curvature can promote the formation of smaller toroids even when the majority of the molecule is not curved. In another study, the introduction of a G-rich sequence into plasmid DNA was shown to decrease the size of toroidal condensates by 22%, but apparently by a different mechanism from that reported here, as the G-rich insert was shown to lack static curvature.[4b]

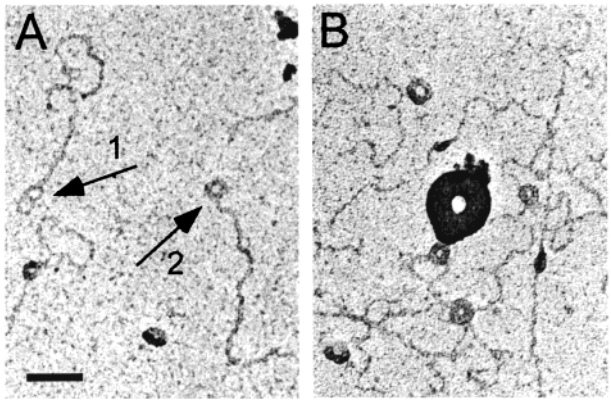

Figure 3. (A) Transmission electron micrograph of partially condensed DNA polymers which contain four tandem copies of the 173 bp A-tract sequence. Arrow 1: Two loops at the end of a partially condensed DNA polymer. Arrow 2: A single circle at the end of a partially condensed DNA polymer. The appearance of the loops near the end of the linear DNA is apparently due to the collinear condensation of the DNA which flanks the A-tract inserts, as these DNA constructs have the A-tract inserts located near the center of the polymers (see Figure 1). (B) Micrograph showing one large toroid among several small toroids. All toroids shown were produced in the same condensation reaction, from the 2961 bp DNA polymer containing four tandem copies of the 173 bp A-tract sequence. (Bar = 100 nm.)

The results presented here support the hypothesis that toroid size can be influenced by the size of the first loop (or loops) upon which DNA is condensed.[5] However, it should be noted that the small toroids were observed in samples placed on EM grids within 5 min of mixing DNA and hexammine cobalt(III). When longer times were allowed between the initiation of DNA condensation and deposition on EM grids (e.g. 30 min), fewer small toroids were observed and larger aggregates appeared. This suggests that the smaller toroids represent a metastable state, which may be unfavorable because of the small DNA radius of curvature. Thermodynamic models have been developed to explain the observed size of toroidal DNA condensates;[11b,12] however, it does not appear that sufficient data presently exist to adequately test the validity of these models. Further studies of toroid nucleation using static loops of various diameters, and their incorporation into polymers of different lengths, may provide the information required to determine what ultimately governs the size of DNA toroids. Such studies are presently underway.

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Supporting Information Available: Experimental procedures for the preparation of DNA and samples for TEM (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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