Cationic silanes stabilize intermediates in DNA condensation

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Abstract In vitro condensation of DNA has been widely studied to gain insight into the mechanisms of DNA compaction in biological systems such as chromosomes and phage heads and has been used to produce nanostructured particles with novel material and functional properties. Here we report on the condensation of DNA in aqueous solutions by cationic silanes, which combine the condensing properties of polyamines with the cross-linking chemistry of silanes. DNA can be reversibly condensed into classical toroidal and rod-shaped structures with these agents. At low silane concentrations DNA forms a variety of looped structures with well-defined characteristics, including flower- and sausage-shaped forms. These structures suggest that at low silane concentrations a DNA-DNA contact in which the strands are at very large angles to each other is stabilized. Changes in these structures observed as a function of silane concentration suggest possible pathways for the formation of toroids and rods.

Key words: DNA condensation; DNA-DNA contact; Toroid; Rod; Cationic silane

1. Introduction

DNA condensation is integral to the formation of biological structures such as chromosomes and phage capsids [1]. In addition, condensed DNA has a number of potentially important technological applications, including the development of non-viral vectors for gene therapy [2–7]. The process of DNA condensation has been studied extensively in vitro, and a wide range of agents are known to promote condensation [8]. Naturally occurring proteins, such as histones and protamine, are involved in DNA condensation in vivo but can also condense DNA in vitro [1,9–12]. Cationic lipids promote the formation of a number of interesting condensed phases of DNA [13–17] and are widely used as transfection agents [18,19]. Polyamines and other small polycations are among the most well-studied in vitro condensing agents. These agents can condense DNA into well-defined, highly ordered structures, in particular toroids and rods [20–24]. Thermodynamic and spectroscopic studies show that the condensation by polycations occurs when approximately 85–90% of the phosphate backbone is neutralized [25,26] and that the process is quite abrupt [25,27,28]. As a result, the condensation process is usually considered as a two-state coil-globule transition [29,30], although there has been a report of several apparent intermediates in spermidine induced DNA condensation on the surface of mica [31]. Recently we found that silicon surfaces treated with cationic silanes could induce the formation of toroids or rods in the absence of soluble multivalent cations [32]. This prompted us to examine the condensing properties of cationic silanes in aqueous solutions.

2. Materials and methods

2.1. Sample preparation

pBR322 DNA (Sigma Chemical Company, St. Louis, MO) was diluted from a stock solution of ~500 μg/ml into purified water (>18 Mohm; MilliQ-UV, Millipore Co., Bedford, MA) to a final concentration of 4 μg/ml. The cationic silane AHA or AEEA (Gelest Inc., Tullytown, PA) was diluted into water to a concentration two times higher than the concentration desired for the DNA treatment. The pH of the water alone was 6.5 and the pH of a 200 μM solution of AHA or AEEA in water was 6.5. Equal volumes (10 μl) of the DNA and silane solutions were mixed and incubated for 10–15 min. This solution was pipetted onto a freshly cleaved mica surface, incubated for 1–2 min, and immediately blown dry with compressed gas (Vari-Air, Peca Products, Janesville, WI).

2.2. AFM imaging

AFM imaging was performed by ambient tapping mode imaging. A Digital Instruments Nanoscope IIIa controller with a Multimode AFM (Digital Instrument, Santa Barbara, CA) was used. Silicon cantilevers (model TESP; Digital Instruments) were used. Imaging conditions were as previously reported [31]. Images are presented with inverted contrast for clarity.

3. Results and discussion

3.1. Complex condensates formed by treatment with cationic silanes

The silanes AHA and AEEA are composed of a trimethoxysilane connected to moieties that resemble putrescine and spermidine respectively (Fig. 1). In neutral aqueous solutions AHA is a divalent cation and AEEA is a trivalent cation. pBR322 DNA treated with AHA at low concentrations and neutral pH, adsorbed to mica and imaged by ambient tapping mode AFM shows a range of highly complex structures (Fig. 2A). One of the most obvious types of structures is the flower-shaped condensate (Fig. 2B) which is composed of a central focus and surrounding loops of DNA that form the 'petals'. These flowers are similar to ones recently reported for spermidine treated DNA [31], although the central focus here appears somewhat larger. A variant of the flower is a condensate in which some or all of the DNA loops are nested (Fig. 2C). Another prominent type of structure are sausage-shaped condensates, in which there are two or more focal points (Fig. 2D, E). In sausages the focal points appear evenly spaced, and the DNA strands between the focal points are well separated. The ends of the sausages usually have loops similar to the ones in flowers, although some are terminated on one or both ends by a focal point. Length measurements of DNA outside the focal points suggest that the majority of conden-

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Abbreviations: AFM, atomic force microscopy; AHA, *N*-(6-aminohexyl)aminopropyl-trimethoxysilane; AEEA, trimethoxysilylopropyl diethylenetriamine

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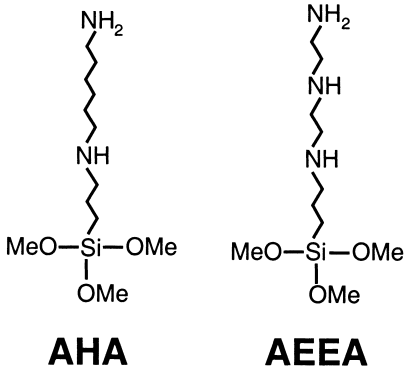

Fig. 1. Chemical structure of the two cationic silanes.

sates formed under the experimental conditions used here are composed of single molecules. Similar results are obtained with linearized pBR322 and lambda phage DNA (not shown). Similar DNA condensates also form with AEEA treatment, although the distribution of structures is different (not shown).

Treatment of pBR322 with relatively high AEEA concentrations (200 μM), at neutral pH, results in the formation of both toroids and rods (Fig. 3). The toroids have a diameter of ~40 nm and thus appear to be identical to those produced by other condensing agents [8]. The silane AHA produces similar results although it is less effective than AEEA at neutral pH. Dilution with water, before or after adsorption to the mica, results in the loss of condensed structures, suggesting that the process is reversible.

The formation of complete toroids and rods is significantly enhanced by lowering the pH during silane treatment to ~4.5, and at that pH complete toroids and rods form readily at 90 μM AHA. We attribute this effect to the cross-linking chemistry of the methoxysilanes. The silane monomers are stable on the time scale of hours in neutral aqueous solutions. However, at low or high pH they hydrolyze rapidly (minutes) to form reactive species that polymerize into silicon gels [33,34]. Lowering the pH of a solution of DNA condensed with AEEA to pH 4.5 for 1–2 min results in stable particles that do not decondense upon dilution with water or treatment with high concentrations of NaCl, as assayed by agarose gel electrophoresis and AFM imaging (not shown). The structure of these silicon-DNA particles and the cross-linking chemistry will be described in more detail elsewhere.

3.2. Possible pathways for toroid and rod formation

Because there is a wide distribution of different structures at each silane concentration it is not possible to rigorously construct a condensation pathway. However, the structures themselves clearly suggest at least one possible sequence of intermediates with increasing silane concentration (Fig. 4). Taking AHA as an example, at 30 μM DNA is relaxed and appears similar to DNA in the absence of the silane. At 90 μM AHA there is looping of the DNA, and flower-shaped structures with a single stable focal point, similar to those recently described for spermidine [31], are common. Highly looped molecules with multiple focal points are also seen. The focal points have either a linear or circular arrangement. As the silane concentration is increased to 180 μM AHA the number of focal points tends to increase, and the focal points are

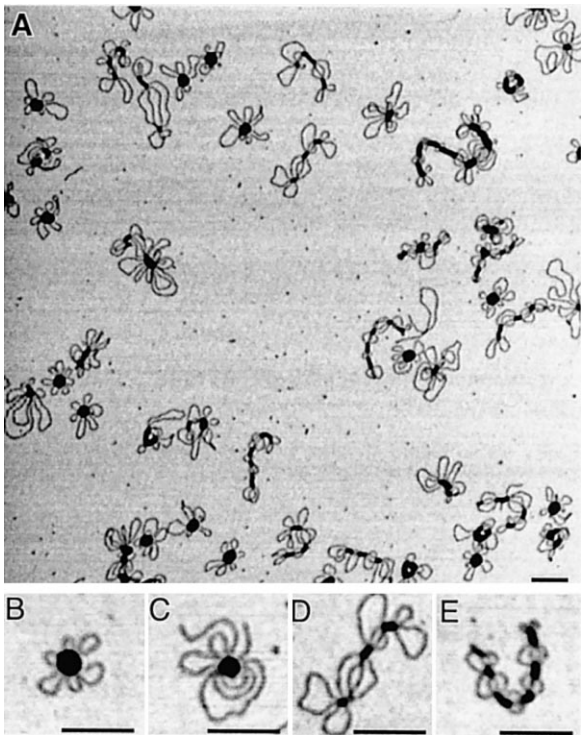

Fig. 2. AFM images of condensates formed by pBR322 and 180 μM AHA at neutral pH. A: Low magnification images show a wide range of complex condensed forms of DNA. B: Flower-shaped condensate with a central focus surrounded by loops of DNA. C: A variant of the flower in which several of the DNA loops are nested. D: Sausage-shaped condensate with three focal points and large end-loops. E: Sausage-shaped condensate with six focal points and small or no end-loops. All scale bars are 125 nm.

enlarged into short rod-like domains. As the silane concentration is further increased, it appears that these rod-shaped domains extend until they contact each other, resulting in partial toroids and nearly complete rods.

Note that the data presented in Fig. 4 are derived from moving DNA along the condensation pathway by gradually increasing the concentration of condensing agent. Thus, these intermediates form under conditions that may be very different from the conditions that exist when DNA is treated directly with concentrations of cations above that required to produce complete toroids or rods. Under those conditions the majority of work indicates that condensation occurs in an abrupt two-state process [27–30], although recent Brownian dynamic simulations suggest that racket-shaped intermediates may form ([35]; David R.M. Williams, personal communication). To get more direct insight into condensation in the

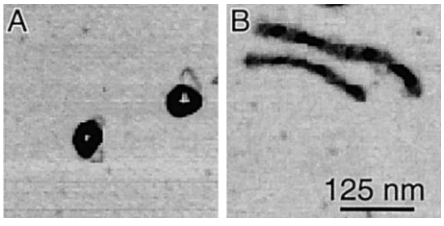

Fig. 3. Treatment of plasmid DNA with 200 μM AEEA results in (A) toroidal and (B) rod-shaped DNA condensates.

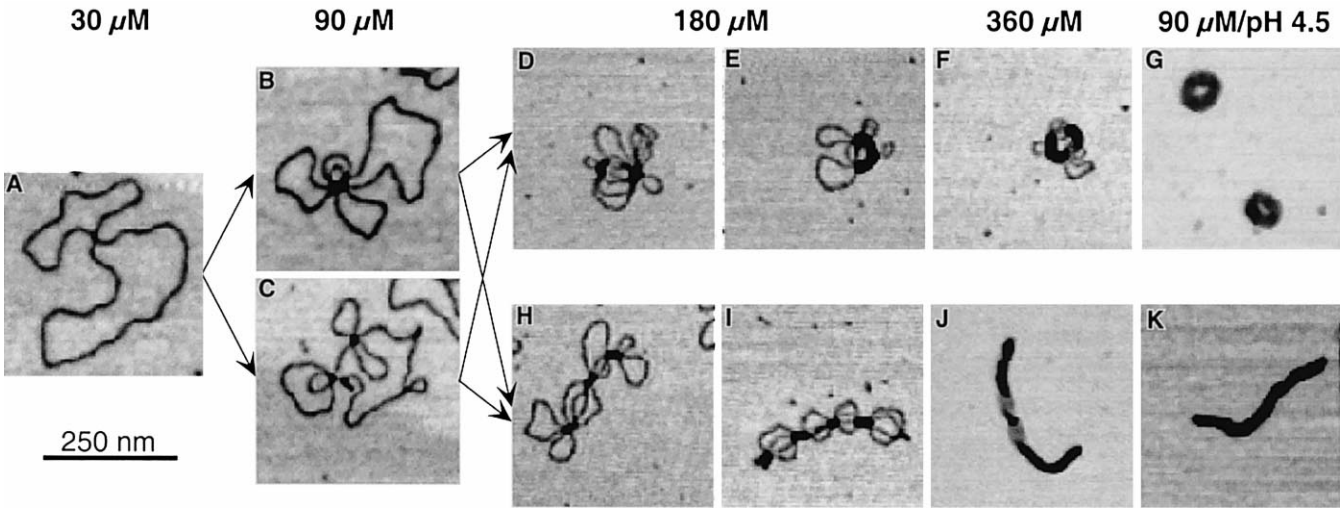

Fig. 4. AFM images of complex structures formed by treatment of DNA with varying concentrations of AHA at pH ~6.5 suggest two possible condensation 'pathways'. At very low (30 μM) AHA concentration the DNA is unstructured. As the concentration of AHA is increased, the DNA shapes become more condensed and increasingly complex. The most distinct feature is the formation of focal points at which several DNA strands meet. Initially there is a single focal point (B and C), followed by structures with multiple regularly spaced focal points (D, H, I and F). The multiple focal points are aligned linearly (H and I) or in a more circular pattern (D and F). Shifting the pH to 4.5 prompts the completion of the condensation to toroids or rods.

presence of high concentrations of condensing agents one would ideally want to examine structures as a function of time, rather than concentration as was done here. However, in our hands this has so far proven difficult.

In addition to the hypothetical pathway described above, the structures of some condensates suggest an alternative pathway. In the majority of the flower foci the center is dome shaped (Fig. 5A). However, occasionally a focal point will have a depression in the middle such that it resembles a toroid (Fig. 5B). Thus there may be a pathway in which toroids grow from a single focal point, rather than the merger of two or more. Similarly, some single focus condensates are highly asymmetric (Fig. 5C, D) suggesting the beginnings of a rod.

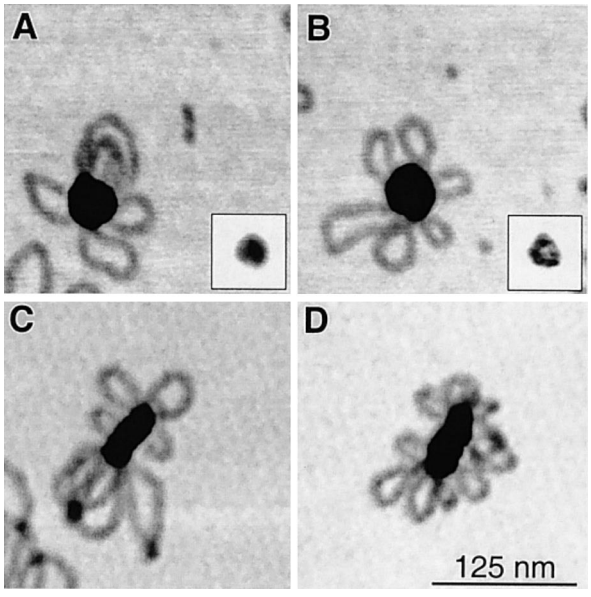

Fig. 5. AFM images of condensates formed by pBR322 and 180 μM AHA at neutral pH that suggest an alternative condensation pathway. A: Flowers typically have dome-shaped focal points. The inset shows a thresholded representation of the center of the focal point, with a filled center. B: Occasionally flowers have a depression in the center, causing the focal point to resemble a toroid. The inset shows a thresholded representation of the center of the focal point, with a depressed center. D, E: Some focal points are highly asymmetric.

3.3. Implications for DNA-DNA contacts

One of the central features of the structures reported is that they appear to contain DNA-DNA contacts in which one DNA strand crosses the second at very large angle (Fig. 6A). This large angle contact is inferred from the shape of loops in the flowers, and the assumption that at the lowest concentrations of silanes at which flowers form the DNA does not bend significantly as it passes through the focus. The alternative is that DNA strands that enter a focal point are sharply kinked, an energetically very costly conformation (Fig. 6B). One interpretation of the data is that there is a critical concentration of certain multivalent cations that stabilize large angle DNA-DNA contacts (Fig. 6A) and a higher critical concentration that stabilizes parallel (or nearly so) DNA-DNA contacts (Fig. 6C). The higher critical concentration would be that required to produce fully condensed toroids and rods [25,26]. Complex structures such as sausages could then arise from overlap of these two contact regimes.

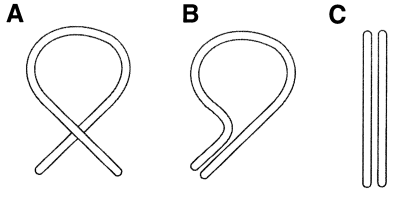

Fig. 6. Schematic of two types of DNA-DNA contacts. A: The organization of DNA in flowers and sausages suggests that there are stable DNA-DNA contacts in which the strand crosses at a large angle. B: To form a loop without a large angle contact would require an energetically costly sharp bend in the DNA. C: At high concentrations of multivalent cations contacts along the length of two DNA strands are stabilized [25,26].

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