Enhanced Resistance of DNA Nanostructures to Enzymatic Digestion

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Statement of Purpose: DNA nanotechnology is increasingly used to create well-defined functional structures. Numerous 2-D and 3-D objects have been built entirely from DNA, in addition to proof-of-concept devices. While DNA structures have potential as vehicles for gene and drug delivery, the ability to avoid rapid degradation *in vivo* is needed. For discrete DNA nanostructures with dimensions much smaller than 50 nm, enzymatic recognition might be quite different from that of linear DNA. Here we report the enhanced stability of DNA tetrahedra with respect to degradation by various enzymes, including DdeI restriction enzyme, DNase I and nucleases that are present in serum.

Methods: DNA sequences used in this study are listed in ref [1]. *Assembly of tetrahedra*: All DNAs were purchased from Integrated DNA Technology (IA) and diluted in TM buffer (10 mM Tris, 5 mM MgCl2) to a final total concentration of 0.8 μM. Solutions were heated at 95 °C for 5 minutes, followed by rapid cooling to 4 °C using a thermocycler (Bio-Rad, CA). *Enzymatic digestion and serum stability*: All enzymes were purchased from New England Biolabs (MA). In the case of DdeI digestion, 0.4 μM of assembled T1 and T2 DNA tetrahedra were first ligated by T4 DNA ligase overnight, followed by the addition of 10 U of Exo III to remove any unligated strands and free ends. The mixtures were then re-assembled using a thermocycler, and 0.07 μg of either tetrahedra DNA or linear DNA were incubated with 2 U of DdeI (30.7 molar ratio enzyme:substrate) for 1 h at 37 °C. For DNase I digestion, 0.2 U of DNase I (0.77 molar ratio enzyme:substrate) was used with incubation at 37 °C for the indicated times. For serum incubation, 11 uL of fetal bovine serum (ATCC, VA) were added to 100 μL of 0.8 μM solution of tetrahedra and linear DNA, and incubated at 37°C and 5 % CO_2. Each sample was taken after desired time of incubation. *Gel electrophoresis*: Solutions of digested mixtures were run on a 12% denaturing PAGE containing 8 M urea in 1x TAE buffer. Native gels were synthesized without addition of formamide and urea. Bands were visualized with Gelstar staining (Lonza, Switzerland) and analyzed using ImageJ software.

Results: We began by comparing digestion patterns between DNA tetrahedra and linear DNA structures. Following the design of Goodman *et al.* [2],we assembled a tetrahedron (T1) having edges of 20 bp in length, or about 7 nm, containing a single centrally-located DdeI restriction site (Fig. 1). To examine the effect of site placement within the pyramid, we designed a variant of the above 20 bp tetrahedron having its DdeI site located adjacent to a vertex (T2). Subsequent incubation of unligated T1 and T2 with DdeI showed no difference when compared to linear controls, suggesting that DdeI can access the site or distort unligated tetrahedra sufficiently to be active. Then, we ligated the nicks in T1 and T2 to form covalent circular strands. While DdeI degrades ligated T1 to yield multiple linear fragments, there are no such species for ligated T2. This indicates that protection is due to the combination of site location and a covalently closed nanostructure. We next examined the resistance to non-specific enzymatic degradation. Upon Digestion with DNase I, unligated T1 is gradually digested by DNase I, but more slowly than L3. Analyzing the band intensities shows that T1 has a decay time constant nearly three times greater than L3. Towards more closely mimicking physiological conditions, we incubated unligated T1 and its linear counterparts in the presence of 10% fetal bovine serum (Figure 2). In this complex mixture of nucleases and other proteins, T1 is significantly more stable than either L3. From fits of the data to first-order kinetics , the decay time constants differ by nearly a factor of fifty: 0.8 h for L3 and 42 h for T1.

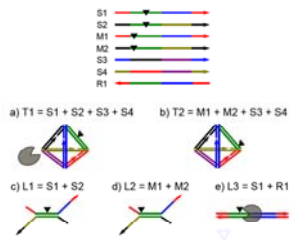

Figure 1. Schematic of DNA strands and self-assembled structures

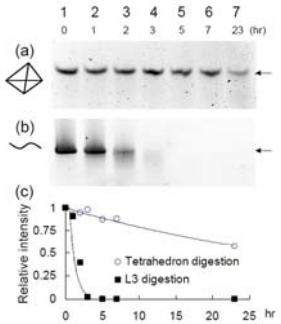

Figure 2. Denaturing PAGE of products from non-specific digestion by 10% FBS (a) unligated tetrahedron (b) L3 (c) Band intensities are well-described by first-order decay.

Conclusions: Due to their small size and unique shape, several DNA tetrahedra are resistant to the action of specific and non-specific nucleases. Such enhanced stability is a key requirement for DNA nanostructures to be useful as delivery vehicles.

References: [1] Keum JW, Chem Commun 2009, in press
[2] Goodman RP, Science 2005, 310, 1661.