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1. Introduction

Topology-specific DNA structures, such as small loops,1 supercoils,2 knots,3 and catenated mini and maxicircles,4 occur in numerous instances in vivo. Polycatenanes can be found in the kinetoplast DNA, a part of the mitochondrial DNA of certain protozoa, consists of a network of thousands of minicircles with 0.5-10 kb and dozens of maxicircles with 20-40 kb.4 Synthetic molecules with interlocked units were achieved in macromolecular chemistry decades ago,5,6 and is the current topic of interest in structural DNA nanotechnology. Interlocked-DNAs, such as DNA catenanes, are of special interest for the construction of molecular switches, motors,7 and logical devices8.

2. Synthesis of topologically-interlocked minicircle DNAs inside a DNA origami frame

By considering the potential of the minicircle and interlocked DNAs, we have designed and characterized the formation of DNA rotaxane and catenane inside a frame-shaped DNA origami (Figure 1).9,10 This origami frame contains four ss-loop connectors (denoted as A, B, C & D) that serve as attachment sites for the rotaxane linear duplex or functions as one of the two rings for the catenane when base-paired with complementary strand. The rotaxane linear duplex contains 67 bp with additional 32 bases long ss-overhangs at each end for attachment with the loop regions of the origami. The rotaxane and catenane rings were prepared by using six different ssDNAs (total length: 183 bp), all phosphorylated at the 5'-end. Intrinsically bent AT-tracts were used to induce the curvature of the ring.11,12 The formation of the interlocked structures was characterized by agarose gel electrophoresis (AGE) using fluorophore-labelled DNA, and also by atomic force microscopy (AFM). As designed, we have successfully prepared the top rotaxane and catenane inside the origami frame (Figure 1, AGE images). The average formation yields before and after ligation were respectively 14 and 31% for top rotaxane, and 42 and 62% for catenane. This indicated clearly that the ligation of the nicks in the rings is necessary to stabilize the interlocked structures. Next, the formed structures were directly visualized by AFM which clearly revealed the linear duplex and ring of the rotaxane, and their attachment between the connectors A-B. The catenane is also formed as designed, and the smaller (loop-A, 64 bp) and the larger (183 bp) rings are clearly observed. The formation yields estimated from the AFM images were 30 and 59% respectively for top rotaxane and catenane, being similar to the AGE results. The outer diameters of the top rotaxane and catenane rings estimated from the AFM images were 25 and 24 nm, respectively, which agree well with the theoretical value of ~24 nm. The rotaxane structure was also prepared at the bottom position between the connectors C-D (42% yield), and at both top and bottom positions (dual rotaxane, 60% yield).

3. Probing nucleosomal DNA topology and DNA-protein interactions

Besides their presence in vivo, DNA minicircles have been used to probe the topological features of
DNA and DNA-protein interactions. The energetic and steric limits of DNA gyrase were probed using DNA minicircles and was shown to be capable of introducing two negative supercoils into a minicircle of length 174 bp. Further, the minicircles with 75 and 86 bp were also used to mimic the nucleosome-induced DNA curvature in the absence of histones for the process of retroviral integration. The HIV-1 integrase reaction was enhanced 5-10 and 15-20 fold respectively in the absence and presence of the LEDGF/p75 integration cofactor for the minicircles when compared to linear DNA of the same length and sequence. This indicated the importance of DNA topology such as curvature; additionally, the cofactor activates integration into nucleosome-like curved DNAs even in the absence of histones. Here, we have utilized the synthesized topologically-interlocked nano-assemblies to probe the DNA topology and DNA-protein interactions by the restriction reactions (Figure 2). For comparison, we have adapted the free-linear duplexes and the duplexes attached inside the origami with relatively less flexibility, and free- or topologically-interlocked minicircles inside the origami. Restriction enzymatic reactions were chosen as they were often used to probe the protein (e.g. transcription factors) accessibility to promoter DNA in chromatin in the methods such as “restriction enzyme protection assay” as proxies for transcription factors. The enzyme PstI has single scission site in the linear duplex of rotaxane and the ring of catenane, while XbaI has a single site in both the rotaxane and catenane rings. At first, the steady state reactions were probed on the interlocked structures inside origami. Though, the enzymatic reactions were proceeded, the estimated reaction yields were as low as 36% to as high as 57%. The double digestions yielded higher reactions of 60-71%, but the reactions never went completion. The time- and enzyme concentration-dependent digestion reactions were also carried out on the interlocked-structures. The reactions proceeded slowly and steadily, and the obtained maximum yields were ≤30%. Further, the analysis was also carried out on the linear duplex and rings in the absence of origami. Except the free-linear duplex, all tested structures were reluctant for the restriction digestion, indicating that the topological features of DNA, such as flexibility, curvature, and groove orientation, play a major role in DNA-protein interactions. Our results by using the histone-free artificial DNA structures are consistent with the investigations carried out on nucleosomal DNAs and revealed the critical influence of DNA topology, similar to the one in nucleosomal DNA, on the DNA-protein interactions.

4. References

Collaboration Works

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Presentations

Rajendran A., Park S., Nakata E., Kwon Y., Osheroff N., Morii T., Enzymatic reactions on topologically-interlocked DNA structures inside a DNA origami frame, The 100th CSJ Annual Meeting, Tokyo University of Science, Tokyo, 2020.3.22-25