様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

令和　６ 年　 ６ 月 ２５ 日現在

機関番号：１４３０１
研究種目：基盤研究(C)（一般）
研究期間：2021～2023
課題番号：２１Ｋ０５２７４

研究課題名（和文）Retroviral integration into topologically-interlocked DNAs to probe the role of DNA structure and screen viral inhibitors

研究課題名（英文）Retroviral integration into topologically-interlocked DNAs to probe the role of DNA structure and screen viral inhibitors

研究代表者

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交付決定額（研究期間全体）：（直接経費）　　3,200,000円

研究成果の概要（和文）：我々は、フレーム状のDNA折り紙の中にトポロジー的にかみ合ったDNAを作成することで、DNA構造がDNA-タンパク質相互作用にどのような影響を与えるかを探った。制限酵素を用いて、DNAトポロジーの重要性を調べた。また、トポイソメラーゼのようなトポロジー特異的タンパク質をこれらの構造体上でテストし、様々な条件下での相対的不安定性を観察した。われわれは、酵素的および化学的方法を用いて、これらの構造を安定化させた。現在、DNA-タンパク質相互作用におけるDNAトポロジーの役割をさらに理解するために、ヌクレオカプシドタンパク質とレトロウイルス統合体との相互作用を研究している。

研究成果の学術的意義や社会的意義
Knowledge obtained on the role of DNA topology is useful to understand the biological process taking place on topologically constrained DNA structure in nucleosomes. Stability improvement methods developed are useful for synthesizing stable DNA nanomaterials for drug delivery and virus inhibition.

研究成果の概要（英文）：We explored how DNA structure impacts DNA-protein interactions by creating topologically interlocked DNA minicircles within a frame-shaped DNA origami. Using restriction enzymes, we found that interlocked minicircles were more resistant than linear DNAs, highlighting the importance of DNA topology. We also tested topology-specific proteins like topoisomerase on these structures, observing their relative instability under various biological conditions. To proceed with DNA-protein interaction studies and drug screening, we stabilized these structures using enzymatic and chemical methods. Our stabilization techniques improved the origami's stability under diverse biological conditions. Preliminary results suggest our platform's potential for analyzing viral proteins such as those from HIV. The current work involves investigating the interaction between nucleocapsid proteins and retroviral integration to further understand DNA topology's role in DNA-protein interactions.

研究分野：Nucleic Acids Chemistry and DNA Nanotechnology

キーワード：DNA origami　DNA nanotechnology　Structural biology　Nucleic acids chemistry　DNA-protein interaction

１．研究開始当初の背景

Retroviruses possess distinctive traits in their replication process that are characterized by two pivotal stages: 1) reverse transcription and 2) integration.[1] Reverse transcription leads to the formation of a ds-DNA replica of the viral RNA. The integration involves inserting the viral DNA copy into the genome of the host cell. The initial enzymatic steps of integration are orchestrated by integrase, an enzyme encoded by the virus. Integration can take place at various sites within the host genome, with certain chromatin areas being favored. Multiple factors, including interactions between host-virus proteins and DNA-proteins, influence the process of integration selectivity. [1] Notably, at the nucleosome level, both DNA and histones play roles in determining integration selectivity, although the specific contributions of each component, such as the impact of structural features of nucleosomal DNA on viral DNA integration, remain unclear. The nucleosomal core consists of around 145-147 bp DNA strands, divided into two gyres of approximately 72 bp each. [2] This results in distinct structural characteristics, including fixed rotational orientation and DNA curvature, when compared to free-linear DNA. Previous studies overlooked the significance of DNA curvature, indicating the need for a versatile approach to investigate the influence of DNA structural features on retroviral integration. Therefore, our research aimed to elucidate the role of DNA structure/topology in the integration process and leverage this understanding to shed light on DNA-protein interaction, and also the development and screening of antiviral medications targeting the integrase.

２．研究の目的

The aim of this research is to replicate the topological structure of the nucleosome by synthesizing topologically interlocked DNA minicircles[3] within a frame-shaped origami. [4] Building on the potential of these minicircles and interlocked DNAs, the objective is to design, fabricate, and characterize the formation of DNA rotaxane and catenane structures within the frame-shaped DNA origami. Despite previous efforts in creating interlocked structures using ssDNAs, dsDNAs, and DNA origami, their practical applications have been limited. Additionally, the exploration of biomolecular interactions, particularly DNA-protein interactions, using interlocked minicircles remains largely uncharted territory. Therefore, the nano assemblies generated in this study will serve as tools for probing DNA topology and DNA-protein interactions through retroviral integration reactions and other protein/enzymatic reactions. To facilitate comparison, the research involves investigating free-linear duplexes and duplexes with reduced flexibility attached inside the origami, alongside free or topologically interlocked minicircles also within the origami structure.

３．研究の方法

The research methods employed in this study encompass the utilization of DNA minicircles, which are present in various biological contexts such as kinetoplast DNA, and are considered promising elements for developing functional nanodevices. These minicircles have been previously employed to investigate DNA-protein interactions.[5] One notable aspect of minicircle DNAs is their ability to mimic the structural characteristics, including curvature and rigidity, of nucleosomal DNA, enabling the study of DNA-protein interactions in the absence of histone proteins. For instance, a minicircle containing 75 base pairs closely emulates the DNA curvature found in a nucleosome. Additionally, DNA structures exhibit topology-specific features, such as small loops (e.g., protein-constrained DNA loops), supercoils, knots, and catenated mini and maxicircles (e.g., kinetoplast DNA), which occur frequently in vivo and are currently a focus of interest in structural DNA nanotechnology. Interlocked DNAs, including DNA rotaxanes and catenanes, hold particular significance for constructing molecular switches, motors, and logical devices. Furthermore, the complexity of rotaxanes and catenanes has been enhanced by employing the DNA origami technique. As for the experimental technique, high-speed atomic force microscopy (HS-AFM)[6] and various other biochemical techniques are used in this study.

４．研究成果

In this study, we aimed to probe the role of DNA structure/topology in DNA-protein interactions. For this purpose, the topologically interlocked DNA minicircles, such as DNA rotaxane and catenane, inside a frame-shaped DNA origami were synthesized and characterized by HS-AFM and other biochemical techniques. After careful optimization of the experimental conditions, the topologically interlocked structures were obtained in good yields (Figure 1, left panel).[7] Before the retroviral integration, at first, we probed the DNA-protein interactions using the restriction enzymes. Interestingly, the minicircles in the free and the topologically interlocked forms exhibited relatively higher resistance when compared to free linear DNAs of the same or similar sequence and length. Similar results were also obtained when enzyme concentration-dependent and kinetic analyses were carried out. This indicated the importance of DNA topology on DNA-protein interactions.

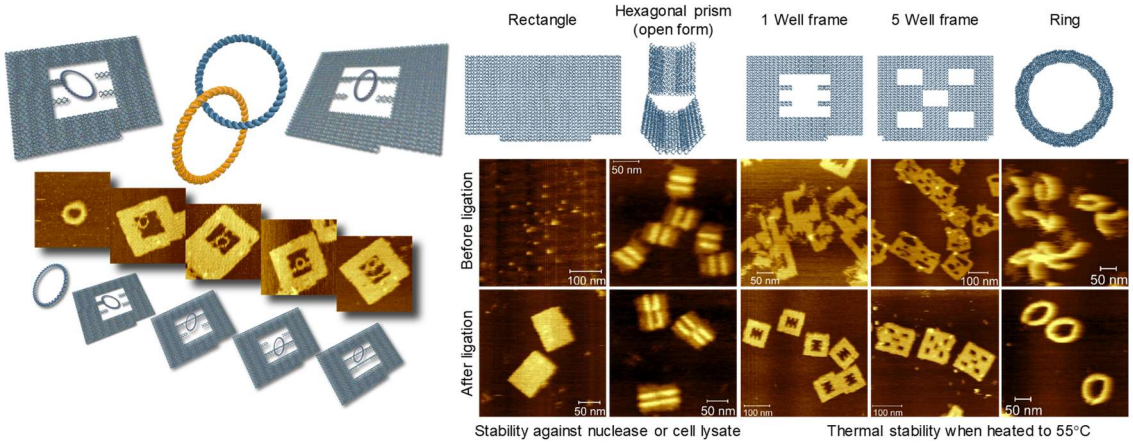

Figure 1. Left: Schematics and HS-AFM images of the DNA minicircle, frame-shaped DNA origami and topologically interlocked DNA rotaxane and catenane inside the DNA origami frame. Right: Stability enhancement of various 2D and 3D origami under thermal treatment, against endonuclease, and in cell lysate.

Next, we also tested the DNA topology-specific proteins, such as topoisomerase, on these structures. During this study, we noticed that these interlocked minicircles and also the DNA origami frame are relatively unstable under various environments and biological conditions. Thus, before the investigation of the DNA-protein interactions any further and drug screening, it was necessary to stabilize these structures. We then developed two near quantitative ligation methods for the native backbone linkage at the nicks in origami: 1) a cosolvent dimethyl sulfoxide (DMSO)-assisted enzymatic ligation and 2) enzyme-free chemical ligation by CNBr.[8, 9] Both methods achieved over 90% ligation in 2D origami structures, only the CNBr method resulted in ~80% ligation in 3D origami structures, while the enzyme-alone yielded 31-55% (2D) or 22-36% (3D) ligation. Only the CNBr method worked efficiently for 3D origami and DMSO-assisted enzymatic ligation resulted in very low ligation efficiency. The CNBr-mediated reaction was completed within 5 min, while the DMSO method took overnight to complete the rection. Ligation by these methods improved the structural stability up to 30ºC, stability during the electrophoresis and subsequent extraction, and against nuclease and cell lysate (Figure 1, right panel). These methods are straightforward, non-tedious, and superior in terms of cost, reaction time, and efficiency. Thus, our stabilization method indicated that origami can now be used under diverse biological conditions with much better stability. Our preliminary results indicated that we could use our platform for the analysis of viral proteins (such as the viral proteins from HIV virus). We are now working on the interaction of nucleocapsid proteins and retroviral integration to further investigate the DNA topology on the DNA-protein interactions.

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５．主な発表論文等

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１．著者名 A. Joseph, A. Rajendran, A. Karithikeyan, B.G. Nair	４．巻 28
２．論文標題 Implantable Microfluidic Device: An Epoch of Technology	５．発行年 2022年
３．雑誌名 Current Pharmaceutical Design	６．最初と最後の頁 679-689
掲載論文のDOI（デジタルオブジェクト識別子） 10.2174/1381612827666210825114403	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

１．著者名 Rajendran Arivazhagan、Krishnamurthy Kirankumar、Park Seojeong、Nakata Eiji、Kwon Youngjoo、Morii Takashi	４．巻 28
２．論文標題 Topologically‐Interlocked Minicircles as Probes of DNA Topology and DNA‐Protein Interactions	５．発行年 2022年
３．雑誌名 Chemistry-A European Journal	６．最初と最後の頁 e202200108
掲載論文のDOI（デジタルオブジェクト識別子） 10.1002/chem.202200108	査読の有無 有
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１．著者名 Arivazhagan Rajendran, Kirankumar Krishnamurthy, Amulya Giridasappa, Eiji Nakata, Takashi Morii	４．巻 49
２．論文標題 Stabilization and structural changes of 2D DNA origami by enzymatic ligation	５．発行年 2021年
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掲載論文のDOI（デジタルオブジェクト識別子） 10.1093/nar/gkab611	査読の有無 有
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１．著者名 Zhengxiao Zhang, Eiji Nakata, Huyen Dinh, Masayuki Saimura, Arivazhagan Rajendran, Kazunari Matsuda, Takashi Morii	４．巻 27
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掲載論文のDOI（デジタルオブジェクト識別子） 10.1002/chem.202103304	査読の有無 有
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1．著者名 Arivazhagan Rajendran, Shiwei Zhang, Takashi Morii	4．巻 28
2．論文標題 Functional nucleic acids-protein complexes: Application to fluorescent rebionucleopeptide sensors	5．発行年 2022年
3．雑誌名 Handbook of Chemical Biology of Nucleic Acids, Springer Singapore, Eds: N. Sugimoto	6．最初と最後の頁 1-20
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3．雑誌名 Small Methods	6．最初と最後の頁 2300999
掲載論文のDOI（デジタルオブジェクト識別子） 10.1002/smtd.202300999	査読の有無 有
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４．発表年 2021年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

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