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研究課題名（和文）Development of new method for screening anticancer drugs that target topoisomerases by using DNA origami

研究課題名（英文）Development of new method for screening anticancer drugs that target topoisomerases by using DNA origami

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研究成果の概要（和文）：The supramolecular assemblies of the topologically interlocked components inside a DNA origami were constructed. Such assemblies of the functional structures are promising in the fields of molecular switches, motors, sensors, and logic devices.

研究成果の概要（英文）：The supramolecular assemblies of the topologically interlocked components inside a DNA origami were constructed. Such assemblies of the functional structures are promising in the fields of molecular switches, motors, sensors, and logic devices.

研究分野：　DNA nanotechnology

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１．研究開始当初の背景

The DNA topoisomerases (Topos) regulate the DNA topology such as overwinding or underwinding that arises due to the intertwined nature of the double helical structure of DNA.[1] These enzymes also play important roles in various biological processes such as replication, transcription, recombination, and chromosome condensation and segregation. During the DNA replication and transcription, overwinding of the DNA duplex occurs. If this overwinding is not relaxed, it eventually stops the functions of the enzymes involved in replication process. Topos control these topological conditions by transiently cleaving the phosphodiester bond, which generates a Topo-DNA cleavage complex. Once the winding stress is resolved, the enzyme-mediated DNA break is resealed. This process is critical for the healthy cells to survive and function normally, and failure to reseal the DNA break can ultimately lead to cell death. Topos involve in step-by-step processes such as binding of Topo to DNA, ATP driven strand passage, strand cleavage by Topo, formation of Topo-DNA cleavage complex, religation of cleaved DNA, and catalytic cycle after DNA cleavage/enzyme turnover. All these reaction steps are of great interest as potential targets for the development of anticancer drugs targeting Topo enzymes.[2]

２．研究の目的

Despite the development of various Topo-inhibitors, the mechanisms of action of these anticancer drug molecules are not well known. For instance, it is not well understood at which step of the enzyme reaction is inhibited by a particular drug molecule. Also, typical methods such as ethidium bromide assay, to measure the topoisomerase inhibitory activity are not suitable for real-time observation of the reaction. To understand the Topos reaction and the mechanisms of the inhibitors, it is necessary to develop a versatile method. Thus, the purpose of this research is to develop a novel single molecule method, to screen the Topo-inhibitors and elucidate the mechanism of action of these drug molecules. Further, the real-time analysis of the Topo enzymes and their inhibitors are investigated.

３．研究の方法

In this work, we have utilized the “scaffolded DNA origami”[3-5] structure as a novel scaffold for the preparation of topologically-interlocked structures that can aid for the analysis of Topo reactions and drug screening. In 2006, “scaffolded DNA origami” method - the folding of DNA strand to create almost any arbitrary two- and three-dimensional nanostructures, was developed by Rothemund.[3] Since then it was successfully utilized for the nanopatterning of transition metals, various nanoparticles, several kinds of proteins, virus-like particles, and other functional components into deliberately designed arrangements.[6-7] It was also applied for the analysis of various reactions and functions at single-molecule level.[8] Further, they act as templates for the growth of nanowires, aid in the structural determination of proteins, and provide new platforms for genomic applications.[9] These structures were also used for the delivery of drugs. To the best of our knowledge, DNA origami nanostructures were not used for the screening of any drug molecule. Thus, by considering the potential of DNA origami, it is of great interest at the current situation that we have utilized these nanostructures for the formation of the topologically constrained DNA structures, and analyze the function of DNA topology specific proteins such as Topo enzymes, and further to investigate the inhibition mechanism of the protein reactions by drug molecules. As for the experimental techniques, in addition to several biochemical techniques, we have used the state-of-the-art high-speed atomic force microscopy (HS-AFM) for the direct and real-time analysis. Regarding the target structures for the Topo reactions, we have constructed the topologically-interlocked DNA catenane- and rotaxane-like structures inside a frame-shaped DNA origami.

４．研究成果

As the Topo enzymes target the topologically constrained DNAs, the mechanically-interlocked supramolecular DNA assemblies can be considered to be the potential targets to investigate the Topo functions and their inhibitors.[10] Topologically interesting structures such as Borromean rings, catenanes, and knots have already been prepared by using DNA.[11] Also, the complexity of the catenane[12] and rotaxane[13] structures were increased by constructing them by the DNA origami method.[3-5] However, the fabrication of the duplex DNA catenanes and rotaxanes to the relatively larger and complex DNA nanostructures such as DNA origami has not yet been realized. These nanomolecular assemblies have potential applications such as the functional components for molecular switches and motors, novel platforms for the investigation of the function of proteins, analysis of protein inhibitors, and so on. Recently, I have been collaborating with the research groups of Prof. Takashi Morii (IAE,

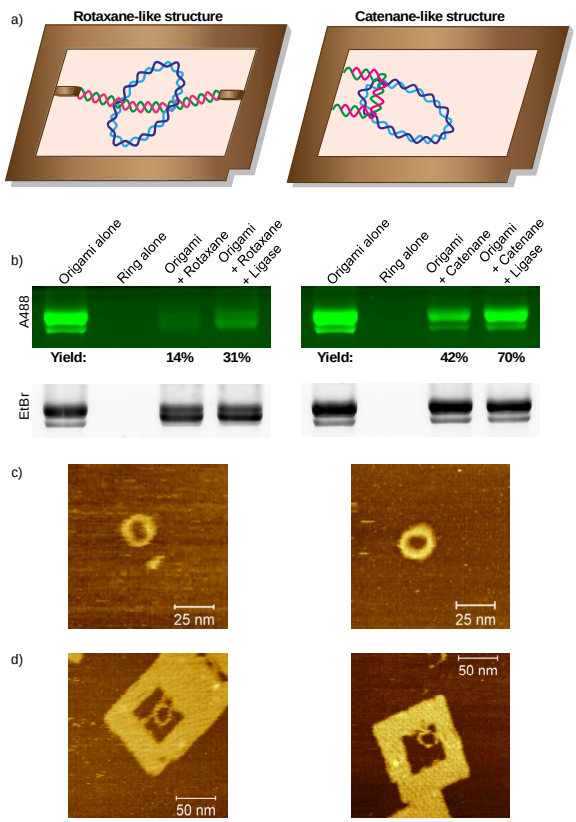

Figure 1. (a) Schematic illustrations of the DNA rotaxane (left) and catenane (right) inside a frame-shaped DNA origami is shown. (b) The confirmation of the formation of topologically-interlocked DNA rotaxane and catenane structures inside the origami by agarose gel electrophoresis. The estimated formation yield values are shown at the bottom of the gels. The yield of the ligated samples were always higher than the non-ligated samples, indicating the higher stability after ligation. (c) AFM images of the rotaxane/catenane ring structure alone. The outer diameter of the ring is about 26 nm. (d) AFM images of the rotaxane (left) and catenane (right) structures inside a DNA origami frame. The numbers in the images indicate the scale bar.

Kyoto University) and Prof. Youngjoo Kwon (Ewha Womans University) for the nanofabrication of the topologically interlocked supramolecular assemblies, to express and purify the Topo enzymes, synthesize the Topo-inhibitors and also to use their experimental techniques and facilities.

Here, we have developed a nanotechnological method by the combination of scaffolded DNA origami[3-5] and HS-AFM[14-22] for the screening of Topo-inhibitors. As for the substrates for the Topo reactions, we have constructed topologically-interlocked DNA catenane- and rotaxane-like structures inside a frame-shaped DNA origami (Figure 1a). The formation of the DNA origami frame and the insertion of the catenane- and rotaxane-like structures were successfully characterized by agarose gel electrophoresis (Figure 1b) and HS-AFM (Figure 1c). To increase the stability of these functional structures, the nicks in these structures were sealed by using T4 DNA ligase. The ligation was also confirmed by the thermal treatment of these structures, where the ligated samples were stable at high temperature incubation while the non-ligated samples failed to keep the folded structures. The ligation at both 4°C and room temperature were performed and both the temperatures lead to the same results. The experimental conditions such as the amount of salt, annealing temperatures, concentration of the DNA strands were optimized. The presence of NaCl did not improve the formation yield of these structures, while it slightly decreased the yield. The purification and quantification methods to get rid of the excessive staples and unbound catenane/rotaxane rings were also established. The yield of the rotaxane and catenane-like structures inside the origami was estimated to be 32 and 68%, respectively. To increase the yield of the rotaxane-like sturctures inside a DNA origami, we have introduced two formation sites inside the origami. In this case, the yield of the target structure was increased to 62%. AFM images indicated that the formed structures had the same dimensions with that of the original design. We have also investigated the stability of the DNA origami frame and the catenane/rotaxane ring structures in the presence of various kinds of Topo inhibitors. Both the origami and the DNA ring are stable against the Topo inhibitors for several hours at room temperature. This indicated that the DNA origami based analysis of Topo inhibitors could be successfully carried out. In addition, the topologically-interlocked structures are stable against the phenol/chloroform/isoamyl alcohol extraction, ethanol precipitation, and lyophilization. Thus, these structures can be prepared, concentrated or freeze dried, stored and used later when they are required. We are now investigating the Topo reactions on these functional structures. After successful investigation of the Topo reactions, these structures will be used for the screening of Topo inhibitors.[23-27] Initially, the commercially available Topo-inhibitors will be screened. Later, the method will be used for the newly

synthesized Topo-inhibitors. Such a screening will be carried out by the direct and real-time characterization methods such as HS-AFM and fluorescence imaging. In addition to the Topo-inhibitors, these nanostructures will also be used for the analysis of various enzymatic reactions, and protein analysis. Apart from the Topo and other enzymatic/protein reactions and inhibitor screening, the fabrication of the topologically-interlocked structures within a DNA origami nanostructure are also promising in the fields of molecular switches, motors and robots, sensors, and logic devices to carry out the automated functions.[4]

＜引用文献＞

[1] C. A. Austin, K. L. Marsh, R. A. Wasserman, E. Willmore, P. J. Sayer, J. C. Wang, L. M. Fisher, *J. Biol. Chem.*, **1995**, *270*, 15739-15746.

[2] C. Wu, T. Li, L. Farh, L. Lin, T. Lin, Y. Yu, T. Yen, C. Chiang, N. Chan, *Science*, **2011**, *333*, 459-462.

[3] P. W. K. Rothemund, *Nature*, **2006**, *440*, 297-302.

[4] A. Rajendran, M. Endo, H. Sugiyama, *Curr. Protoc. Nucleic Acid Chem.*, **2012**, *48*, 12.9.1-12.9.18.

[5] A. Rajendran, E. Nakata, S. Nakano, T. Morii, *ChemBioChem*, **2017**, *18*, 696-716.

[6] H. T. Maune, S. Han, R. D. Barish, M. Bockrath, W. A. Goddard III, P. W. K. Rothemund, E. Winfree, *Nat. Nanotechnol.*, **2010**, *5*, 61-66.

[7] E. Nakata, F. F. Liew, C. Uwatoko, S. Kiyonaka, Y. Mori, Y. Katsuda, M. Endo, H. Sugiyama, T. Morii, *Angew. Chem. Int. Ed.*, **2012**, *51*, 2421-2424.

[8] A. Rajendran, M. Endo, H. Sugiyama, *Angew. Chem. Int. Ed.*, **2012**, *51*, 874-890.

[9] S. M. Douglas, J. J. Chou, W. M. Shih, *Proc. Natl. Acad. Sci. U. S. A.*, **2007**, *104*, 6644-6648.

[10] J. L. Nitiss, E. Soans, A. Rogojina, A, Seth, M. Mishina, *Curr. Protoc. Pharmacol.*, **2012**, *57*, 3.3.1-3.3.27.

[11] T. L. Schmidt, A. Heckel, *Nano Lett.*, **2011**, *11*, 1739-1742.

[12] D. Han, S. Pal, Y. Liu, H. Yan, *Nat Nanotechnol.*, **2010**, *5*, 712-717.

[13] J. List, E. Falgenhauer, E. Kopperger, G. Pardatscher, F. C. Simmel, *Nat. Commun.*, **2016**, 7:12414.

[14] A. Rajendran, M. Endo, H. Sugiyama, *Chem. Rev.*, **2014**, *114*, 1493-1520.

[15] A. Rajendran, M. Endo, K. Hidaka, H. Sugiyama, *Angew. Chem. Int. Ed.*, **2014**, *53*, 4107-4112.

[16] A. Rajendran, M. Endo, K. Hidaka, H. Sugiyama, *J. Am. Chem. Soc.*, **2013**, *135*, 1117-1123.

[17] A. Rajendran, M. Endo, K. Hidaka, P. L. T. Tran, J.-L. Mergny, R. Gorelick, H. Sugiyama, *J. Am. Chem. Soc.*, **2013**, *135*, 18575-18585.

[18] A. Rajendran, M. Endo, H. Sugiyama, *Angew. Chem. Int. Ed.*, **2012**, *51*, 874-890.

[19] A. Rajendran, M. Endo, Y. Katsuda, K. Hidaka, H. Sugiyama, *J. Am. Chem. Soc.*, **2011**, *133*, 14488-14491.

[20] T. Ando, T. Uchihashi, S. Scheuring, *Chem. Rev.*, **2014**, *114*, 3120-3188.

[21] A. Rajendran, M. Endo, K. Hidaka, M.-P. Teulade-Fichou, J.-L. Mergny, H. Sugiyama, *Chem. Commun.*, **2015**, *51*, 9181-9184.

[22] A. Rajendran, K. Hidaka, N. Shimada, A. Maruyama, H. Sugiyama, *Chem. Commun.*, **2014**, *50*, 8743-8746.

[23] A. C. Ketron, W. A. Denny, D. E. Graves, N. Osheroff, *Biochemistry*, **2012**, *51*, 1730-1739.

[24] H. Kwon, C. Park, K. Jeon, E. Lee, S. Park, K. Jun, T. M. Kadayat, P. Thapa, R. Karki, Y. Na, M. S. Park, S. B. Rho, E. Lee, U. Kwon, *J. Med. Chem.*, **2015**, *58*, 1100-1122.

[25] J. L. Nitiss, E. Soans, A. Rogojina, A. Seth, M. Mishina, *Curr. Protoc. Pharmacol.*, **2012**, *57*, 3.3.1-3.3.27.

[26] B. Pogorelcnik, M. Janezic, I. Sosic, S. Gobec, T. Solmajer, A. Perdih, *Biorg. Med. Chem.*, **2015**, *23*, 4218-4229.

[27] J. H. Lee, T. J. Wendorff, J. M. Berger, *J. Biol. Chem.*, **2017**, *292*, 21011-21022.

５．主な発表論文等

〔雑誌論文〕（計 2 件）

1) A. Rajendran,* E. Nakata, S. Nakano, T. Morii,* Nucleic-acid-templated enzyme cascades, *ChemBioChem*, **2017**, *18*, 696-716.
DOI: 10.1002/cbic.201600703

2) A. Rajendran,* N. Shigi, J. Sumaoka, M. Komiyama,* One-pot isolation of a desired human genome fragment by using a biotinylated pcPNA/S1 nuclease combination, *Biochemistry*, **2018**, *57*, 2908-2912.
DOI: 10.1021/acs.biochem.8b00202

〔学会発表〕（計 6 件）

1) A. Rajendran, S. Park, E. Nakata, Y. Kwon, T. Morii, Supramolecular assemblies of mechanically interlocked components inside a DNA origami frame, *The 98th Annual meeting of The chemical society of Japan*, Nihon University, Japan, 20-23 Mar. **2018**. Oral: 4D7-01.

2) A. Rajendran, S. Park, E. Nakata, Y. Kwon, T. Morii, DNA rotaxane and catenane inside a DNA nanostructure, *The 2nd International Symposium on Biofunctional Chemistry*, Kyoto University, Japan, 14-16 Dec. **2017**. Poster: P-83.

3) A. Rajendran, S. Park, E. Nakata, Y. Kwon, T. Morii, Construction of topologically interlocked DNAs inside a DNA scaffold, *The 44th International symposium on nucleic acids chemistry and 1st Annual meeting of Japan society of nucleic acids chemistry*, Tokyo University of Science, Japan, 14-16 Nov. **2017**. Oral: 1O-05.

4) A. Rajendran, DNA nanotechnology and it's medicinal applications, *2017 International Conference on Environmental Medicine and the 14th Symposium of the Frontiers of Biomedical Sciences*, Kaohsiung Medical University, Taiwan, 10-11 Nov. **2017**. Keynote speech.

5) A. Rajendran, S. Park, E. Nakata, Y. Kwon, T. Morii, Assembly of DNA rotaxane and catenane inside a DNA origami frame, *The 11th Symposium on bio-relevant chemistry*, University of Tokyo, Japan, Sept. **2017**. Poster: 2PB-75.

6) A. Rajendran, S. Park, E. Nakata, Y. Kwon, T. Morii, Topologically interlocked DNA nanostructures inside a DNA origami frame, *The 97st Annual meeting of The chemical society of Japan*, Keio University, Japan, 16-19 Mar. **2017**. Oral: 2C4-17.

〔その他〕

ホームページ等

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