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研究課題名（和文）Precise coating of DNA origami nanostructures by 'template polymerization' method

研究課題名（英文）Precise coating of DNA origami nanostructures by 'template polymerization' method

研究代表者

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交付決定額（研究期間全体）：（直接経費）　　3,000,000円

研究成果の概要（和文）：DNAナノ構造の安定化と機能化を目指し、主鎖にグアニジニウムイオンGu +、側鎖にアジド基を含む線形モノマーを合成しました。 合成した線形モノマーとナノ構造を混合すると、モノマーのGu+がDNAのリン酸基と多価的な塩橋を形成し、表面電荷が中和されたために線形モノマー／ナノ構造複合体は沈殿するという問題が起きました。そこでより柔軟な骨格をもつデンドリマー型の分子を新たに合成し、複合体の溶解性を改善しました。このデンドリマー型の分子の性質をさらに調べるため、40塩基対の長さのDNAを用いて体系的な調査を行いました。予備実験からは、この分子を用いてDNAにタンパク質を固定化できることがわかっています

研究成果の学術的意義や社会的意義
我々はDNAナノ構造の被覆および機能化のため、DNAへの接着部位としてグアニジニウムイオン Gu+を有する分子の開発に取り組んでいます。 より簡単な構造である40塩基対のDNAを使用し、合成した分子とDNA間に働く相互作用について体系的な調査を行いました。DNAナノ構造はドラッグデリバリーシステムにおける輸送体として実用化が期待されていますが、血中で酵素による分解を急速に受けるため、DNAナノ構造の表面を被覆し生物学的な機能を付与することが必要です。 我々が開発してきた、Gu+含有分子を用いたDNAナノ構造の被覆・機能化の技術は、低毒性かつ血中で安定な輸送体の開発に貢献する可能性を有しています

研究成果の概要（英文）：For stabilization and functionalization of DNA-nanostructures, two macromonomers comprising guanidinium ion (Gu+) at their main chain or side chain, and azide units were synthesized. Upon mixing with a DNA origami 6-helix bundle, the macromonomers successfully adhered to the phosphate groups of exposed helices of DNA, however, the solubility of resulting macromonomer/origami conjugate was poor. The solubility issue was partly solved upon using the macromonomer with a more flexible backbone and benzophenone (BP) motif. We systematically investigated how the monomer interacts with DNA using a 40-base pair DNA. Our preliminary data also suggested that the glue coated 40-base pair DNA can be further functionalized with proteins.

研究分野：polymer

キーワード：DNA nanostructure　Drug delivery system　Template polymerization　Adhesion　peptide conjugation

１．研究開始当初の背景 (background at the beginning of the study)

'DNA origami' involves the programmed folding of single stranded deoxyribonucleic acid (DNA) into ordered nanostructures using smaller staple strands. In principle hydrophilic or hydrophobic drugs can be encapsulated during the origami process and released site specifically. However, the DNA-nanostructures disassemble in a lower salt concentration and in the presence of enzymes. Hence, the structural integrity of nanostructures in physiological conditions must be addressed for the practical applications of DNA origami nanostructures as drug delivery system.

２．研究の目的 (purpose of research)

(a) Design, synthesis and fabrication of 'polymer coat' for DNA origami nanostructures to enhance its stability in physiological conditions.

(b) Design and development of a post-modification method for easy functionalization of biologically active targeting ligands to DNA nanostructures.

(c) Proof-of-concept study of ligand functionalized DNA nanostructures using *in vitro* cancer cell lines (*e.g.* Hep3B)

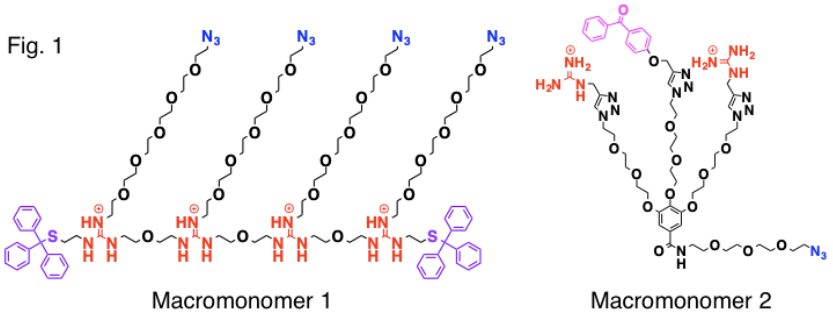
３．研究の方法 (research method)

- Synthesize Guanidinium ion (Gu^+)-based monomers consisting multiple Gu^+ ions on its main chain or side chain and a polymerizable group at their termini.
- Investigate adhesion, charge neutralization and polymerization behavior of synthesized monomer with 20–200 base pair double stranded DNA.
- Preparation and characterization of DNA-origami nanostructures by transmission electron microscopy (TEM)
- Optimization of 'template polymerization' using Gu^+ monomers and DNA bundle
- Proof-of-concept study using simple DNA origami nanostructures such as a 6-helix bundle.
- Stability check of polymer coated DNA 6-helix bundle using serum proteins and blood.
- Cellular uptake study of polymer coated DNA bundle using cancer cell lines.

４．研究成果 (research results)

DNA is an attractive biomolecular motif for constructing precise nanostructures, for instance, DNA-origami methods to prepare nanostructures. The next step in this field is to stabilize the nanostructure and to develop a methodology of functionalizing such DNA nanostructures for practical applications. We synthesized two macromonomers comprising guanidinium ion (Gu^+) and anchoring azide units for stabilization and functionalization of DNA-nanostructures (Fig. 1). We have used either

Fig. 1



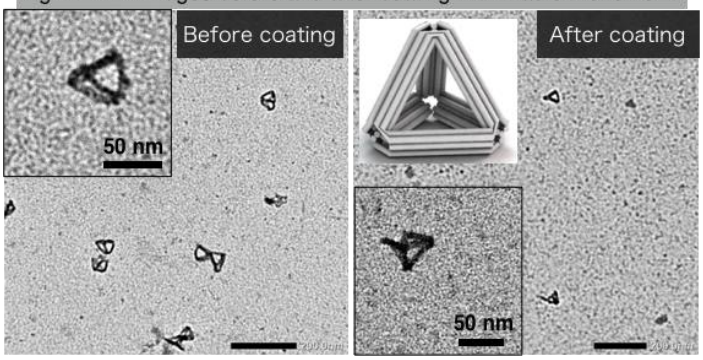
double stranded DNA (40–200 base pairs) or DNA nanostructures such as 6-helix bundle, cuboid, box and tetrahedron.

In a proof-of-concept study, upon mixing with a DNA origami 6-helix bundle, macromonomer 1 with multiple Gu^+ ions in the main chain and azide in the termini of ethylene glycol side-chains adhered to the phosphate groups of solution exposed phosphate groups of DNA via 'salt-bridge' interactions. However, oxidative polymerizations of the DNA-adhered macromonomers tend to precipitate in the reaction buffer, possibly due to increased hydrophobicity of macromonomer/origami conjugate.

Interestingly the macromonomer 2 with Gu^+ ions in a dendritic arm, a photoreactive benzophenone (BP) moiety and a terminal azide group adhered to a 6-helix bundle after photoirradiation as evidenced by the inhibited migration of the bundle under a gel electrophoresis, likely due to the charge neutralization of DNA via 'salt-bridge' interaction between Gu^+–phosphate group. Our current understanding suggest that BP may act as an anchoring unit via DNA intercalation.

We also found that the structural integrity of nanostructure maintained after complexation with macromonomer 2. We observed under transmission electron microscopy (TEM) the intact structure of a tetrahedron DNA origami, after mixing with macromonomer 2 followed by photoirradiation (Fig. 2).

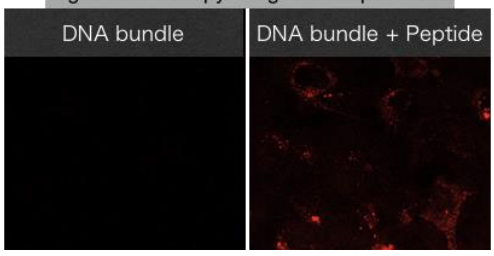
Fig. 2: TEM images before and after coating with macro monomer 2



Due to the presence of azide in macromonomer 2, we were able to functionalize DNA nanostructure via an azide-alkyne click reaction. We mixed the DNA 6-helix bundle after coating with macromonomer 2 with a fluorescent dye appended dibenzyl cyclohexane (DBCO). Electrophoresis data suggested the presence of dye functionalization onto the bundle due to the click reaction between DBCO and azide on the coated bundle.

We then prepared alkyne appended peptide and functionalized to a DNA bundle. Upon incubation with Hep3B cells, we found slightly increased uptake compared with non-coated bundle (Fig 3).

Fig. 3: Microscopy images of Hep3B cells



We investigated systematically how the macromonomer 2 interact with DNA using a 40-base pair DNA. When mixed with DNA, the photoreactive macromonomer 2 preferentially bound to the double strands by the action of the benzophenone unit as an intercalator. Macromonomer 2 on DNA could also adhere to proteins and immobilize them upon photoirradiation. Although, further studies are required, we hope this preferential interaction of macromonomer 2 on DNA could be translate into nanostructures in order to functionalize certain specific region or to make a janus-type functional DNA material.

５．主な発表論文等

〔雑誌論文〕　計2件（うち査読付論文　2件／うち国際共著　2件／うちオープンアクセス　0件）

１．著者名 Ai Kohata, P. K. Hashim, Kou Okuro, Takuzo Aida	４．巻 141
２．論文標題 Transferrin-Appended Nanocaplet for Transcellular siRNA Delivery into Deep Tissues	５．発行年 2019年
３．雑誌名 Journal of the American Chemical Society	６．最初と最後の頁 2862－2866
掲載論文のDOI（デジタルオブジェクト識別子） 10.1021/jacs.8b12501.	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 P.K Hashim, Julian Bergueiro, E.W. Meijer, Takuzo Aida	４．巻 0
２．論文標題 Supramolecular Polymerization: A Conceptual Expansion for Innovative Materials	５．発行年 2020年
３．雑誌名 Progress in Polymer Science	６．最初と最後の頁 101250
掲載論文のDOI（デジタルオブジェクト識別子） https://doi.org/10.1016/j.progpolymsci.2020.101250	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

〔学会発表〕　計1件（うち招待講演　0件／うち国際学会　1件）

１．発表者名 P.K Hashim, Ai Kohata, Kou Okuro, Takuzo Aida
２．発表標題 Coating and Functionalization of DNA-origami Nanostructure for Biomedical Applications
３．学会等名 Annual Meeting of the Chemical Society of Japan（国際学会）
４．発表年 2018年～2019年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

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