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科学研究費助成事業　　研究成果報告書

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研究課題名（和文）バイオマスDNAの加熱処理によるカーボンナノ材料の作製と特性評価

研究課題名（英文）Preparation and characterization of carbon nanomaterials prepared by thermal treatment of biomass DNA

研究代表者

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研究成果の概要（和文）：　本研究は、廃棄バイオマス由来DNAの利用に注目し、機能性カーボンナノ材料の作製技術の開発を目的としている。従来、多糖類系バイオマスからマテリアル作製に関する研究が多かったが、本研究はバイオマスDNAを対象に、熱水処理、マイクロ波処理、および熱分解法を用いて機能性材料を作製を行った。水熱法やマイクロ波法により、DNAから蛍光ナノ材料を合成し、ドーピングによる蛍光特性を制御することに成功し、DNA由来バイオドットは重金属センシングに応用できることを示した。本研究の成果により、熱処理技術を用いたDNAバイオマス由来マテリアルの範囲が拡大し、持続可能なバイオマス処理技術を確立できた。

研究成果の学術的意義や社会的意義
本研究は、魚の白子など廃棄バイオマス系DNAを利用した機能性カーボンナノ材料の製造技術を開発した点で学術的に重要ある。従来の多糖類バイオマス利用研究の枠を広げ、DNAを対象にした水熱処理、マイクロ波処理、熱分解法を体系的に確立して、DNAバイオマスの応用可能性を広げた。本研究は、廃棄バイオマスDNAを有用資源として活用する新技術を提供し、持続可能な資源利用に貢献している。また、DNA由来の蛍光ナノ材料を用いた水銀など重金属検出は、環境汚染監視や公衆衛生保護に役立つ可能性があり、廃棄物の高付加価値化を通じて循環型社会の実現に寄与している。

研究成果の概要（英文）：　Robust methods of waste biomass utilization are increasingly demanded by modern society. While most past efforts were focused on waste polysaccharide biomass, only a few studies considered the utilization of biomass DNA for the preparation of functional materials. In this study, we developed upcycling technologies for hydrothermal, microwave-assisted, and pyrolytic processing of biomass DNA to prepare functional carbon nanomaterials.
In particular, we developed synthetic methods for fluorescent nanomaterials (biodots) from DNA by hydrothermal and microwave methods and succeeded in controlling the fluorescent properties of biodots by doping. DNA-derived biodots were applied for heavy metal detection applications.
The results of this project extended the scope of DNA biomass-derived products prepared by thermal processing thus representing a valuable addition to the existing sustainable biomass processing technologies.

研究分野：高分子化学

キーワード：DNA　廃棄物リサイクル　水熱合成　マイクロ波合成　炭素ナノ材料　センシング

１．研究開始当初の背景

Utilization of renewable biomass feedstock is an area of increasing scientific interest and the target of intensive global research. Biomass is utilized either for (i) a direct conversion to energy through combustion or (ii) for the preparation of various functional materials. Available in large quantities biomass is mainly represented by polysaccharides (cellulose, chitin, etc.), fats, proteins, and nucleic acids. The majority of reports found in the literature focus on carbohydrate feedstocks due to their availability and easiness of processing. In contrast, there have been almost no studies on DNA utilization as a biomass. During the past decade, the availability and application of natural DNA materials have been dramatically broadened and the key objective of the proposed research was to make clear the possibility of utilizing waste-derived DNA for the preparation of functional materials using the thermal processing method.

２．研究の目的

While most past efforts were focused on polysaccharides, only a few studies considered the utilization of nucleic acids for the preparation of functional materials. DNA is ubiquitous on Earth and can be extracted in tons quantities from a waste of marine industry such as fish milt, which makes DNA a very attractive sustainable resource for functional materials preparation. The aim of the proposed research was (i) to systematically investigate the process of nucleic acids thermal decomposition by hydrothermal and pyrolytic methods, (ii) to clarify structure and properties of the products and mechanism of their formation from DNA, and (iii) to test potential applications in fields of bioimaging, photocatalysis, and sensors.

３．研究の方法

The synthetic phase focuses on the preparation of carbon nanomaterials, namely carbon dots (CD) and graphitic carbon nitride (g-C3N4) from DNA feedstocks. Two methods are utilized for the thermal processing: (i) hydrothermal and microwave treatment of nucleic acids and (ii) pyrolytic decomposition of nucleic acids. Mainly, DNA extracted from fish milt was used as a starting material, while RNA, nucleotides and nucleosides were also used for comparison.

４．研究成果

(1) Synthesis of fluorescent nanomaterials (biodots) from DNA by hydrothermal method.

Fluorescent nanoparticles (biodots) of several nm size in diameter were successfully synthesized from polymeric and monomeric nucleic acids: DNA, RNA, nucleotides, and nucleosides using hydrothermal (HT) reaction (**Figure 1A, C**). Spectroscopic studies revealed that the formation of biodots proceeds through the degradation of ribose sugar,

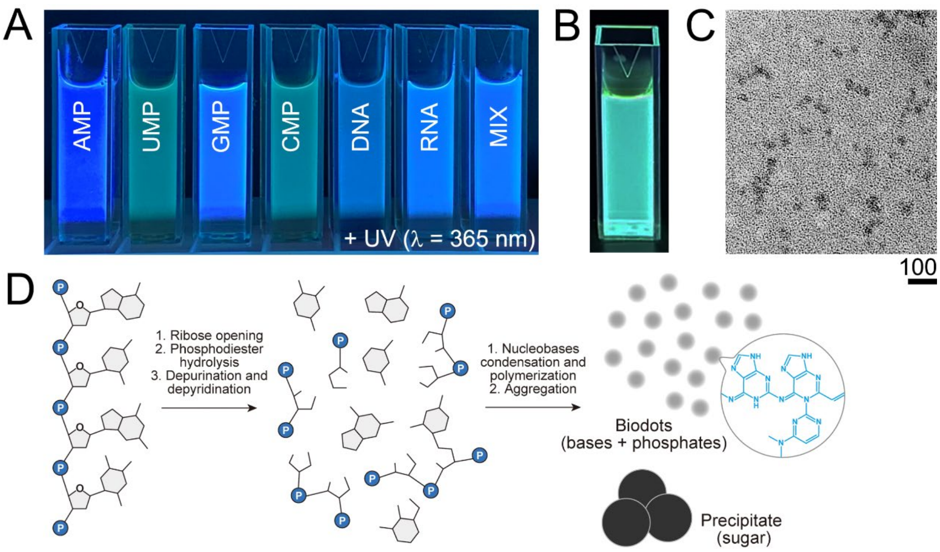

Figure 1. A. Fluroescence of biodots prepared from DNA, RNA and nucleotides (AMP, UMP, GMP, and CMP) under UV light. **B.** Fluroescence of the solution of biodots prepared from DNA and phenilene diamine. **C.** TEM images of DNA-derived biodots. **C.** The mechanism of biodots formation from DNA.

whereas depurination, depyrimidation, and consequent fusion of heteroaromatic nucleobases result in the formation of fluorescent nanoparticles (**Figure 1D**). The chemical structure of precursors affects strikingly optical properties of biodotes In particular, HT treatment of nucleic acids containing purine nucleobases yields over 50 times brighter biodots compared to the precursors containing pyrimidine ones. The fluorescence of nucleic acid biodots is stable in a broad range of pHs and the presence of physiologically relevant cations.

The HT synthesis of DNA biodots using a conventional HT reactor and a microwave reactor under the same conditions was conducted to compare the fluorescent properties of products. The emission wavelengths were similar for both types of reaction products but the biodots prepared using the microwave reactor had 1.5-2-fold higher fluorescence compared to those obtained in the conventional HT heating reaction. This difference originated due to a more homogenous energy transfer in the microwave reactor during biodots synthesis. In addition, microwave heating exhibited lower energy consumption providing additional benefits of energy efficiency and reduced environmental impact.

(2) Multicolor biodots prepared by DNA HT treatment with phenylene diamines. HT and microwave synthesis of DNA-derived fluorescent nanomaterials were further elaborated to control the fluorescence intensity and the emission wavelengths by admixing different nitrogen-containing reagents (urea, aliphatic, aromatic polyamines, etc.). The hydrothermal and microwave synthesis of DNA biodots in the presence of amines via the single-step treatment of DNA-amine mixtures and two-step route were performed. HT and microwave treatment of DNA in the presence of urea and aliphatic amines result in biodots with only blue fluorescence and significantly lower quantum yields (QY) than DNA biodots. In contrast, the hydrothermal reaction of DNA with aromatic diamines (phenylene diamines) resulted in products with green fluorescence (**Figure 1B**) and QY about 4 times higher than that of DNA biodots. The obtained results demonstrated a potential possibility to control the color and fluorescence intensity of DNA-derived nanomaterials by doping with other molecules.

(3) Application of DNA-derived biodots for heavy metal detection. Fluorescent nanoparticles (biodots) synthesized from nucleic acids inherit the high affinity of nucleic acids to Hg^{2+} and, to a lesser extent, to Ag^{+} and Cu^{2+}, which was utilized for the sensing of these heavy metal ions in aqueous solutions, as well as for naked-eye detection of these ions. The fluorescent properties and metal ion sensing characteristics of biodots are notably affected by the chemical structure of the nucleic acid used as a starting material for HT treatment. Fluorescent nanoparticles prepared from individual AMP and GMP nucleotides exhibit a significantly higher fluorescence compared to biodots prepared from UMP, CMP, and DNA. Furthermore, biodots prepared from individual nucleotides show better selectivity to Hg^{2+} ions and a linear dependence of fluorescence on Hg^{2+} concentration, in a broad concentration range. In addition, biodots can be used to make paper-based sensor strips for detecting metal ions in aqueous solutions. Paper strip-based sensors represent a new

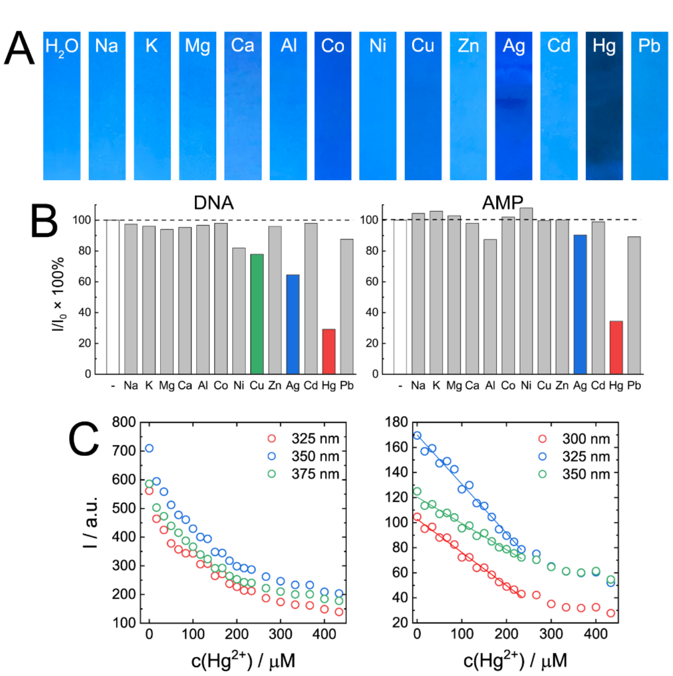

Figure 2. A. DNA biodots-based stripe tests for metal ions. **B.** Quenching of DNA and adenosine monophosphate (AMP) biodots fluorescence by different metal ions. **C.** Dependence of DNA and AMP biodots fluorescence on Hg^{2+} concentration.

strategy for developing simple metal ion indicators for a variety of analytical and environmental applications.

(4) Biochars from DNA. Biochars and hydrocars were prepared from DNA or DNA-derived materials by the pyrolytic treatment at 300-500 °C and hydrolytic treatment at 200 °C. The obtained chars were studied for their adsorption capacities towards typical environmental pollutants (heavy metal ions and pharmaceuticals) by standard batch adsorption experiments. DNA-derived biochars and hydrochars showed low adsorption capacities (below 1 mg/g) towards all studied adsorbents compared to the original DNA and DNA-containing materials and standard adsorbents such as activated carbon. The decrease in the adsorption capacity during thermal treatment of DNA materials was ascribed to the decomposition of nitrogen-containing and other functional groups that served as active centers for binding with the adsorbing molecules. The application of DNA-derived chars as adsorbents was thus shown to be limited. Furthermore, it was concluded that the presence of the phosphate moieties in the DNA prevented the formation of the graphitic carbon structures suggested at the beginning of the study.

(5) Conclusions. Based on the high impact of previous studies reported the "green" synthesis of carbon nanomaterials, the conducted research provided important knowledge on the scope and limitations of DNA biomass-derived products prepared by thermal processing and has a strong potential to be a valuable addition to the existing sustainable biomass processing.

５．主な発表論文等

〔雑誌論文〕　計4件（うち査読付論文　4件／うち国際共著　3件／うちオープンアクセス　3件）

１．著者名 Postnova Irina、Sarin Sergey、Zinchenko Anatoly、Shchipunov Yury	４．巻 4
２．論文標題 DNA-Chitosan Aerogels and Regenerated Hydrogels with Extraordinary Mechanical Properties	５．発行年 2021年
３．雑誌名 ACS Applied Polymer Materials	６．最初と最後の頁 663～671
掲載論文のDOI（デジタルオブジェクト識別子） 10.1021/acsapm.1c01585	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 Wang Maofei、Tsukamoto Masaki、Sergeyev Vladimir G.、Zinchenko Anatoly	４．巻 11
２．論文標題 Metal Ions Sensing by Biodots Prepared from DNA, RNA, and Nucleotides	５．発行年 2021年
３．雑誌名 Biosensors	６．最初と最後の頁 333～333
掲載論文のDOI（デジタルオブジェクト識別子） 10.3390/bios11090333	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

１．著者名 Wang Maofei、Tsukamoto Masaki、Sergeyev Vladimir G.、Zinchenko Anatoly	４．巻 11
２．論文標題 Fluorescent Nanoparticles Synthesized from DNA, RNA, and Nucleotides	５．発行年 2021年
３．雑誌名 Nanomaterials	６．最初と最後の頁 2265～2265
掲載論文のDOI（デジタルオブジェクト識別子） 10.3390/nano11092265	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

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２．論文標題 Adsorptive Removal of Heavy Metal Ions, Organic Dyes, and Pharmaceuticals by DNA-Chitosan Hydrogels	５．発行年 2021年
３．雑誌名 Gels	６．最初と最後の頁 112～112
掲載論文のDOI（デジタルオブジェクト識別子） 10.3390/gels7030112	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 －

〔学会発表〕　計2件（うち招待講演　0件／うち国際学会　2件）

1．発表者名 Wang Maofei、Zinchenko Anatoly
2．発表標題 Assembly, fluorescent properties, and metal ion sensing characteristics of biodots synthesized from DNA, RNA, and nucleotides
3．学会等名 Pacifichem 2021（国際学会）
4．発表年 2021年

1．発表者名 DNA-Chitosan Hydrogels: formation, properties, and environmental applications
2．発表標題 Zinchenko Anatoly、Chan Kayee、Morikawa Kohki、Shchipunov Yury
3．学会等名 7th Congress of Federation of Asian Polymer Societies（国際学会）
4．発表年 2021年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

Sustainable Materials Lab at Nagoya University
https://www.urban.env.nagoya-u.ac.jp/suschem/research.html

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

共同研究相手国	相手方研究機関