Self-Assembly Science Research Section

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1. Introduction

In recent years, DNA origami has emerged as a novel technique for constructing materials ranging from nano to micrometer scale, with sub-nanometer addressability.[1] This technique has been utilized in various applications, including chemical, biological, and materials science. DNA origami has also been used for organizing enzyme cascades, and studies have shown that it can enhance the efficiency and rate of sequential reactions.[2] However, the use of DNA origami for templating biomass-related enzymes is hindered by their poor stability under various conditions. For example, origami materials tend to melt around 50°C when subjected to thermal treatments.[3,4,5] Furthermore, origami materials such as origami cuboid can break even under mild forces, which are applied during structural analysis by force-based methods such as atomic force microscopy (AFM).[6] Biomass often undergoes chemical pretreatments using strong acids or bases to break down the lignin. The biomass product contains several carboxylic acids with a pH of 2 to 2.5. However, origami materials are stable only between pH 4.5-10 but denature at a lower pH. The samples can be stored in pure water for several applications.[8] However, the triangular origami exhibits several defective sites in pure water. Additionally, the origami undergoes digestion against nucleases such as DNase I, which is the most abundant nuclease in blood and plasma, either in vitro or in cell culture medium, and T7 endonuclease I. Most origami synthesis buffers contain 5-20 mM Mg^{2+}, as origami cannot be folded without Mg^{2+}. However, when the folded origami is exchanged into an Mg^{2+}-free buffer, its structural integrity changes depending on its super/globular structure and buffer composition. For example, the 6-helix bundle retains its folded structure when exchanged into Tris, Tris-acetic acid-EDTA (TAE), and phosphate buffers, while the 24-helix bundle remains intact only in Tris buffer. Similar results were observed for a tubular origami, which retained its folded structure when exchanged into the crystallization buffers of various proteins, such as HEPES, PEPES, and 2-(N-morpholino)ethanesulfonic acid (MES). The presence of phosphate buffers and EDTA in TAE can competitively replace the origami-bound Mg^{2+}, thereby destabilizing the structure. The structure-dependent denaturation of origami in low magnesium tissue culture medium was also reported. Regarding other cations, origami is unstable in the presence of K^+, Ca^{2+}, and NH_4^+, which are usual supplements in protein crystallization buffers. Overall, these findings indicate that Mg^{2+} is necessary to ensure the folded structure, while the ionic strengths in physiological conditions are much lower than needed to ensure origami stability. The typical Mg^{2+}, Na^+, and K^+ concentrations in cell culture media are 0.04-0.8, 150, and 5.5 mM, respectively, making this environment unsuitable for origami materials. Not only in biological applications, but also in material applications such as spray coating, low magnesium is required. Therefore, it is essential to develop methods to stabilize DNA origami nanomaterials for diverse applications, especially when dealing with enzymes involved in biomass energy conversion.

2. Approaches to increase the stability of DNA origami nanomaterials

The presence of nicks in the phosphate backbone of staple strands is one of the primary reasons for the instability of origami materials. While increasing the length of the staple strands could improve thermal stability, it could also create practical challenges, such as higher synthetic costs, decreased product yield and purity, and limitations on the maximum length of synthetic oligo DNAs. Several methods have been attempted to address the stability issues of origami materials. Some methods that have been reported include photo-cross-linking by 8-methoxypsoralen[3] and the formation of cyclobutane pyrimidine dimers[7] to introduce covalent linkages. However, these methods are not suitable where native-like DNA is anticipated, as they introduce chemical cross-links to the DNA strands. Also, the irradiation of UV light for 1 to 2 h often leads to DNA damage. Additionally, coating with lipid bilayers, virus capsids, other proteins, cationic polymers, and spermidine has been attempted. In addition to these non-natural treatments, we have demonstrated the optimal conditions for enzymatic ligation of staple nicks in 2D origami. However, this method resulted in only 31-55% ligation efficiency, and thermal stability improved by only 5-20°C,

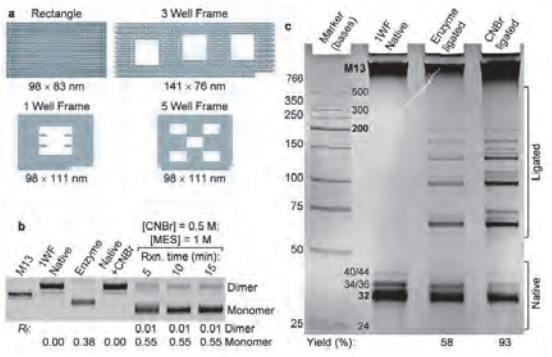

Figure 1. a) DNA origami nanomaterials used in this study. b) Characterization of the chemical ligation by AGE. c) Estimation of the ligation efficiency by PAGE.

depending on the structure. The only other ligation method reported for full-size origami is chemical ligation by *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide (EDC). Despite being an interesting demonstration, this method requires tedious processes. Batch modification of the 3'-end of all the ~226 staples is necessary, amino-modified ddNTPs and terminal deoxynucleotidyl transferase are required in addition to T4 polynucleotide kinase (PNK), and the method also releases isourea as a by-product. Moreover, this method creates an unnatural backbone linkage with an amino group at each ligation site that can be readily cleaved upon treatment with mild acids. Thus, to improve the stability of DNA origami, we have recently performed enzymatic ligation[9] and cosolvent-assisted enhancement in enzymatic ligation. However, these methods do not work for 3D DNA origami and require overnight for the reaction to complete. Thus, we aimed to perform the chemical ligation of DNA origami. To carry out this research, we collaborated with Prof. Takashi Morii's group at the Biofunctional Chemistry Research Section of the Advanced Energy Utilization Division.

3. Chemical ligation of DNA origami nanomaterials

The chemical ligation process was carried out using CNBr on four different types of 2D square lattice DNA origami, including rectangle (Rec),[1] 1 (1WF),[10] 3 (3WF),[11] and 5 well-frame (5WF),[12] as shown in Figure 1a. Agarose gel electrophoresis (AGE) was used to characterize the ligation reaction. As illustrated in Figure 1b, the chemically ligated origami migrated faster compared to the native and enzymatically ligated origami. This clearly indicates that the chemical ligation method is more efficient compared to the other methods mentioned above. Moreover, the chemical ligation reaction was completed within 5 min, highlighting the advantage over enzymatic ligation, which typically requires overnight reaction time for saturation. To optimize the ligation yield, reaction conditions were carefully adjusted. As a result, the highest possible ligation efficiency, exceeding 90%, was achieved under the optimized conditions (Figure 1c). This ligation method not only improves the thermal stability up to 30ºC but also enhances stability during electrophoresis and subsequent extraction, as well as provides resistance against nuclease and cell lysate. In addition to its superior efficiency, this method is straightforward and non-tedious, with a cost advantage over other methods, making it an attractive option.

4. Conclusions

In summary, our study demonstrates the use of chemical ligation as an efficient method for nick ligation in DNA origami nanomaterials. By optimizing the reaction conditions, we were able to ligate staple nicks in origami with near-quantitative yields. This method was successfully tested on four different DNA origami structures. The advantages of using CNBr-mediated ligation include a faster reaction time of just 5 min, along with quantitative reaction yields and native phosphate ligation. Additionally, the ligation of origami using this method enhances its stability against thermal treatments, during electrophoresis and purification, as well as providing resistance against nuclease and cell lysate. Overall, our findings suggest that chemical ligation is a highly effective method for achieving efficient and stable nick ligation in DNA origami, with potential applications in biomass-related enzymes in particular and in nanotechnology, biophysics, and synthetic biology in general.

5. References

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Collaboration Works

Rajendran Arivazhagan，Visvesvaraya Technological University（インド），Stabilization of DNA nanomaterials by enzymatic and chemical methods

Rajendran Arivazhagan，National Institute of Technology，Calicut（インド），DNA nanomaterials for the analysis of single molecular reactions

森井孝，Rajendran Arivazhagan，Vanderbilt University School of Medicine（アメリカ），Topoisomerase 反応の可視化

森井孝，中田栄司，Rajendran Arivazhagan，Ewha Womans University（大韓民国），小分子による酵素機構の解明

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Rajendran Arivazhagan, Scientific Research (C), Retroviral integration into topologically-interlocked DNAs to probe the role of DNA structure and screen viral inhibitors, FY2021-FY2023

2. Others

Chuaychob, Surachada, （公財）ヒロセ財団，トリプレットリピート病の原因となるＲＮＡタンパク質凝集体形成機構の解明と創薬スクリーニング

Publications

A. Rajendran, K. Krishnamurthy, S. Park, E. Nakata, Y. Kwon, T. Morii, Topologically-interlocked minicircles as probes of DNA topology and DNA-protein interactions, *Chem. Eur. J.*, **2022**, e202200108.

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Presentations

A. Rajendran, K. Krishnamurthy, E. Nakata, T. Morii, Cosolvent improves the enzymatic ligation of DNA origami, The 102nd Annual Meeting of the Chemical Society of Japan, Online, 2022.03.23-26.

A. Rajendran, K. Krishnamurthy, E. Nakata, T. Morii, Efficient ligation of nicks in DNA origami, The 49th International Symposium on Nucleic Acids Chemistry, Tokyo University of Science, Katsushika Campus, 2022.11.02-04.