Polymerase incorporation of consecutive ethenoadenosine nucleotides toward the construction of metallo-DNA supramolecules

(*Graduate School of Science, The University of Tokyo*) ○Masahiro Wakano, Silpa Chandran Rajasree, Yusuke Takezawa, Mitsuhiko Shionoya

Keywords: Metal-mediated base pair; 1,N^6-Ethenoadenosine; Enzymatic synthesis; DNA Polymerase; Artificial DNA

Metal-mediated unnatural base pairs, consisting of ligand-type nucleobases and a bridging metal ion, have gained increasing attention for the application in the development of metal-responsive DNA materials.[1] 1,N^6-ethenoadenine (**εA**) is a modified nucleobase that can form a Ag^I-mediated and a Cu^{II}-mediated base pairs (Fig. 1a) inside DNA duplexes.[2] We previously reported that the formation of at least three **εA**–Cu^{II}–**εA** base pairs significantly stabilizes DNA duplexes.[3] This study aimed to develop a new enzymatic method to incorporate multiple **εA** nucleotides into DNA strands under milder conditions to facilitate the application of the **εA**–Cu^{II}–**εA** base pair as metal-responsive molecular switches.

First, we examined the incorporation of an **εA** nucleotide with natural templates by DNA polymerases (Fig. 1b). Dpo IV, a lesion-bypass DNA polymerase, was used because it can extend the strands beyond mismatched base pairs and modified bases. Primer extension experiments were performed in the presence of template strands, each providing a different template base, using 10 equiv of **εA** nucleoside triphosphates (d**εA**TP). Denaturing polyacrylamide gel electrophoresis (PAGE) analysis showed that one **εA** nucleotide was incorporated in about 70% yield after 6 h of incubation when the template base was **T**, but in only 35% yield when the template was **A**. Next, consecutive incorporation of three **εA** was examined using a template strand containing three consecutive **T** bases. When 100 equiv of d**εA**TP was added, more than 70% of the primers were elongated to +3 or more after 48 h of incubation. This result suggests that three **εA** nucleotides were successfully incorporated. Interestingly, the addition of the downstream strand as a blocker was found to inhibit over-elongation (i.e., incorporation of more than three **εA**). This enzymatic synthesis method of **εA**-containing DNA will be applied to the construction of metal-responsive DNA materials.

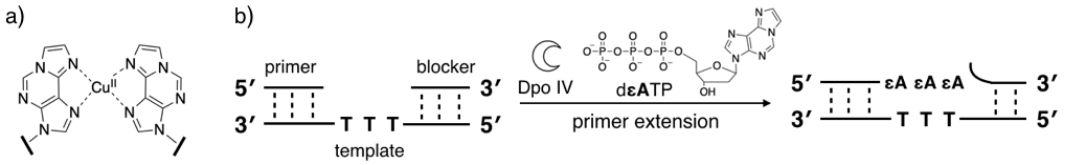

Fig 1. a) Structure of an **εA**–Cu^{II}–**εA** base pair. b) Polymerase incorporation of three consecutive **εA** nucleotides into DNA strands.

1) Y. Takezawa et al., *Chem. Lett.* **2017**, *46*, 622. 2) J. Müller et al., *Inorg. Chem.* **2016**, *55*, 7041. 3) S. C. Rajasree et al., The 101st CSJ Annual Meeting, **2021**, A25-4am-03.