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Ag^{I} Ion Mediated Formation of a C–A Mispair by DNA Polymerases**

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DNA forms a double-stranded structure through the formation of adenine–thymine (A–T) and guanine–cytosine (G–C) Watson–Crick base pairs.[1] The selectivity of the hydrogen bonding between bases is essential for the replication and expression of genetic information. For two decades, the development of an artificial base pair has been an area of research with the goal of expanding the genetic alphabet.[2] Several groups have reported artificial base pairs formed by non-Watson–Crick hydrogen bonding[3] and hydrophobic interaction based on shape complementarity.[4] Some of these alternative base pairs, such as isoguanine–isocytosine,[5] dκ–dX,[6] dZ–dF,[7] dPICS–dPICS,[8] $dImN^{O}$–$dNaO^{N}$[9], and dDs–dPa[10] pairs, were reported to be recognized and incorporated into a primer strand by DNA polymerases.

Metal-mediated base pairs are formed by the coordination of metal ions to natural or artificial bases,[11] and have attracted considerable interest for nanodevices. Recently, Ono et al. reported that Hg^{II} and Ag^{I} ions specifically stabilize the thymine–thymine (T–T) and cytosine–cytosine (C–C) mismatches in oligodeoxynucleotide (ODN) duplexes through the formation of the T-Hg^{II}-T and C-Ag^{I}-C base pairs, respectively (Figure 1 a).[12–14] Also, the formation of a U-Hg^{II}-U base pair in RNA was reported.[15] We focused on the biological relevance of metal-mediated base pairs and discovered that in the presence of Hg^{II} ions, DNA polymerases used thymidine 5′-triphosphate (TTP) to incorporate thymidine at the site opposite a thymine in the template strand and elongated the primer to synthesize a full-length product.[16] Following our discovery, Park and co-workers reported the extension reactions of primer strands that have a T–T or C–C mismatch at the 3′-terminus, in the presence of Hg^{II} or Ag^{I} ions, respectively.[17] Also, an artificial Cu^{II}-mediated base pair recognized by DNA polymerases was reported.[18] However, the Ag^{I} ion mediated incorporation of a deoxynucleotide into a primer strand by DNA polymerases has not yet been reported. The discovery of natural and artificial metal-mediated base pairs recognized by polymerases may increase the possibility of replicating and amplifying artificial metal-containing DNA nanodevices. Herein, we report a primer extension reaction in the presence of Ag^{I} ions.

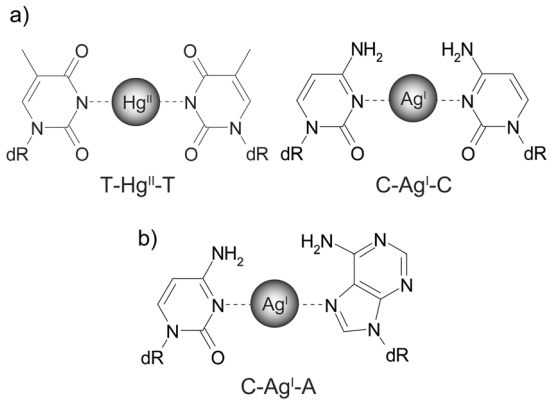

Figure 1. a) Chemical formulas of T-Hg^{II}-T and C-Ag^{I}-C base pairs, and b) a possible structure of the C-Ag^{I}-A base pair. dR = deoxyribose.

The primer extension experiments were carried out using the primed template shown in Figure 2 a with the Klenow fragment (KF) DNA polymerase. In the presence of dATP, dGTP, and dCTP, the extension reactions afforded the full-length product regardless of the presence or absence of Ag^{I} ions (Figure 2 b, lanes 1 and 2). This result shows that the polymerase activity is not inhibited by Ag^{I} ions at the concentration of 30 μM. In the presence of dATP and dCTP, the reaction without Ag^{I} ions was terminated at the site opposite the C residue in the template to yield the 19-mer product (lane 3). In contrast, as Ag^{I} ion concentration was increased (1–50 μM), KF elongated the primer to yield the full-length 24-mer as the major product (lanes 4–9). We also used an extended primed template, which contains two C residues in the single-strand region of the template, KF also afforded the full-length product but with reduced efficiency (see Figure S1 in the Supporting Information). At higher Ag^{I} ion concentrations (500–1000 μM), the elongation was inhibited and the reactions produced weaker bands on the gel, probably because of the aggregation of DNA (see Figure S2 in the Supporting Information). Furthermore, KOD Dash and Taq DNA polymerases also catalyzed this reaction, meaning that this phenomenon is not specific to KF

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Supporting information for this article (experimental details) is available on the WWW under http://dx.doi.org/10.1002/anie.201109191.

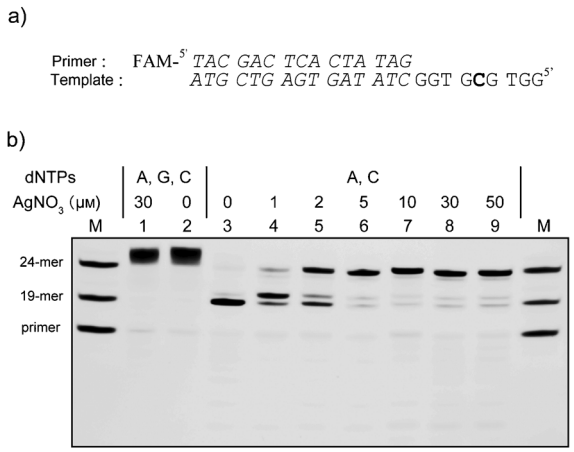

Figure 2. a) Sequences of the template and primer strands. The primer was labeled with fluorescein amidite (FAM) at the 5′ end. b) Effects of Ag^{I} ion concentration on the primer extension reaction by the Klenow fragment (KF). The reaction mixtures (20 μL) containing 100 nM primer, 150 nM template, 20 μM dNTPs, 100 mM NaOAc, 10 mM Tris-AcOH (pH 7.9), 10 mM $Mg(OAc)_2$, 5 mM NH_4Cl, 0.3 units of KF, and 8 μM dithiothreitol (DTT) in the presence or absence of various concentrations of $AgNO_3$ were incubated at 37 °C for 1 h. The reactions were quenched by adding 100 mM DTT (0.5 μL) and a gel loading solution (2.28 μL) containing 8 M urea, 50% sucrose, and 0.2% bromophenol blue, and the mixtures were immediately heated at 90 °C for 10 min. After cooling, the mixtures were analyzed by denaturing 20% PAGE. M indicates markers for the primer, 19-mer and 24-mer.

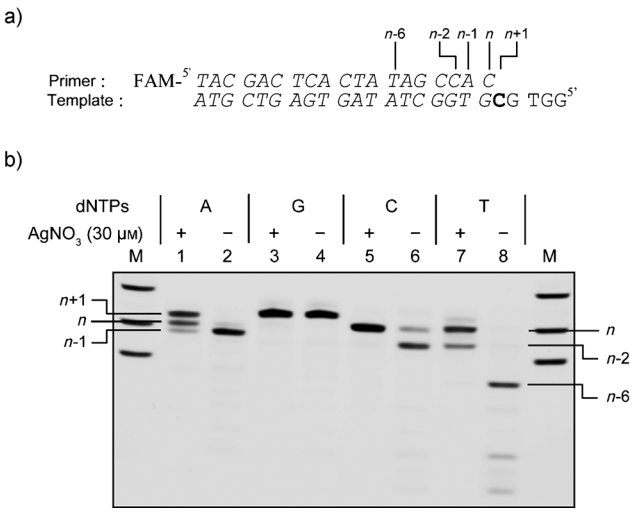

Figure 3. a) Sequences of the template and 5′-FAM-labeled primer strands. b) Single nucleotide insertions at the site opposite the C residue in the template strand were carried out by KF. The reaction conditions are the same as those indicated in Figure 2b. M indicates markers for the 15-mer, primer, and 24-mer.

(see Figure S3 in the Supporting Information). To exclude the effects of the counteranion (nitrate), the reactions were also carried out in the presence of $Ag^{I}(NO_3)$, $Na^{I}(NO_3)$, or $Mg^{II}(NO_3)_2$. Only $Ag^{I}(NO_3)$ promoted the reaction (see Figure S4); thus, we conclude that it is the Ag^{I} ions that mediate the incorporation of cytosine or adenine into the site opposite the C residue in the template by KF.

Next, to confirm the kind of nucleotide incorporated into the site opposite the C residue in the template, single nucleotide insertion reactions were carried out (Figure 3). KF incorporated guanine through the formation of a Watson–Crick G–C base pair to yield the 20-mer $(n+1)$ product regardless of the presence or absence of Ag^{I} ions (lanes 3 and 4). In the absence of Ag^{I} ions, adenine, cytosine, or thymidine were not incorporated into the site opposite the C residue in the template and the primer was degraded by the $3'\rightarrow5'$ exonuclease activity of the enzyme to afford $n-1$, $n-2$, and $n-6$ products, respectively (lanes 2, 6, and 8). The $3'\rightarrow5'$ exonuclease activity of KF competes with the polymerization activity. In the absence of the complementary dNTP (in this case dGTP), the exonuclease domain of KF seems to degrade the primer strand to yield truncated products that have the same base at the 3′-terminus as the dNTP that has been added to the reaction. Unexpectedly, in the presence of Ag^{I} ions, KF misincorporated adenine into the site opposite the C residue in the template (lane 1) to yield the 20-mer $(n+1)$ product together with a trace amount of the 18-mer $(n-1)$ product. However, the enzyme did not misincorporate cytosine into the same site at all (lane 5). Furthermore, even $3'\rightarrow5'$ exonuclease-deficient KF (KF exo$^-$) gave the same results (Figure S5, lanes 5 and 6). These results indicate that when dGTP was absent, KF specifically incorporated adenine into the site opposite the C residue in the template, probably through the formation of C-Ag^{I}-A, a silver(I)-mediated base pair. The composition of the full-length products of the Ag^{I}-promoted reaction was confirmed by MALDI-TOF mass spectroscopy (see Figure S6 in the Supporting Information).

To investigate the effects of other metal ions on the reaction, we performed the primer extension reaction in the presence of Mn^{II}, Fe^{II}, Fe^{III}, Co^{II}, Ni^{II}, Cu^{I}, Cu^{II}, Zn^{II}, Cd^{II}, Au^{I}, Au^{III}, Hg^{II}, Tl^{I}, or Pb^{II}. Figure 4 shows the relative amounts of the full-length product of the reactions catalyzed by KF. Although the reaction was not highly specific to Ag^{I} ions, the Ag^{I} ion-mediated reaction gave much higher yield than any of the other metal-mediated reactions. Some metal ions, such as Mn^{II}, Cu^{I}, and Cu^{II}, did catalyze the reaction but with low efficiency and reproducibility.

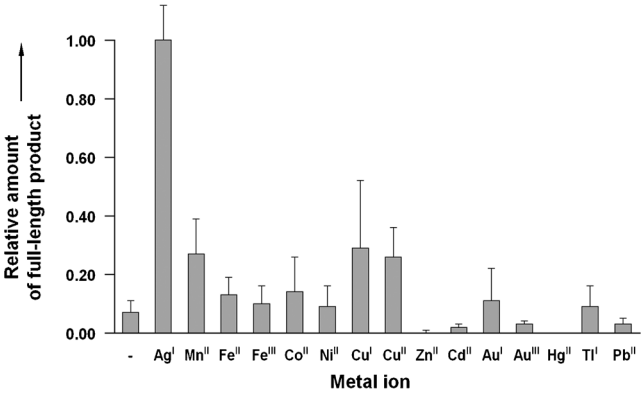

Figure 4. Effects of various metal ions on the primer extension reaction catalyzed by KF. Shown are the amounts of the full-length products of metal ion mediated reactions relative to that of Ag^{I} ion mediated reaction. Values are averages ± the standard deviation determined by at least five independent experiments. The reactions contained 30 μM metal ions and the other conditions are the same as those indicated in Figure 2b.

The molecular basis of the Ag^I-promoted selective incorporation of adenine rather than cytosine is still ambiguous. The UV melting experiments demonstrated that the C–C mismatch-containing duplex was more stable than the C–A mismatch-containing duplex by addition of Ag^I ions (see Table S1 in the Supporting Information). It was reported that when KF exo$^-$ misincorporates nucleotides to form a purine–purine hydrogen-bonded mispair, the incoming dNTP rotates to the *syn* conformation to maintain the C1′-C1′ distance between complementary residues of canonical B-DNA, however, the purine residue in the template is maintained in the normal *anti* conformation.[19] Thus, we investigated the incorporation of cytosine opposite an A residue in the template strand with added Ag^I ions (Figure 5). In the presence of dATP and dCTP, however, the reactions were terminated at the site opposite the A residue in the template strand even in the presence of Ag^I ions (lanes 4–9). This result may suggest that an incoming dATP rotates to the *syn* conformation and coordinates to an Ag^I ion with its Hoogsteen face (Figure 1b) to maintain the C1′–C1′ distance of B-DNA as reported in 1-deazaadenine-containing oligonucleotides.[20] Indeed, the preferential binding of Ag^I-modified 1-methylcytosine to the N7 position of 9-methyladenine was reported.[21] The *anti* to *syn* rotation of the dA residue in oligonucleotides would be energetically more disadvantageous than that of the incoming dATP. Therefore, the Ag^I-mediated stabilization of the C–A mismatch-containing duplex (shown by the UV melting experiments; Table S1) may be compensated by the energy required for the *anti* to *syn* rotation of the dA residue in the template strand.

In conclusion, we demonstrated that KF does not incorporate cytosine into the site opposite a C residue in the template strand even in the presence of Ag^I ions. Instead, adenine was shown to be incorporated opposite the C residue in the template strand by KF in the presence of Ag^I ions, probably through the formation of a silver(I)-mediated C-Ag^I-A base pair. Our findings may open up new possibilities for the discovery of additional metal-mediated base pairs recognized by DNA polymerases, leading to the construction of a metal ion-triggered replicating system and the enzymatic preparation of metal-containing DNA nanodevices.

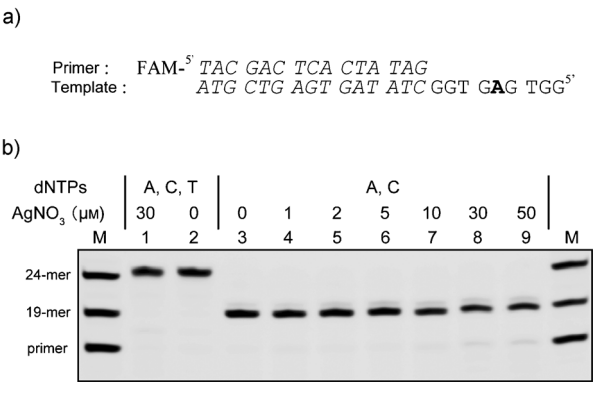

Figure 5. a) Sequences of the template and 5′-FAM-labeled primer strands. b) Effects of Ag^I ion concentration on the incorporation of cytosine opposite adenine in the template by KF. The reaction conditions are the same as those indicated in Figure 2b. M indicates markers for the primer, 19-mer, and 24-mer.

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