Mismatched Base Pairing

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Incorporation of Thymine Nucleotides by DNA Polymerases through T–Hg^{II}–T Base Pairing

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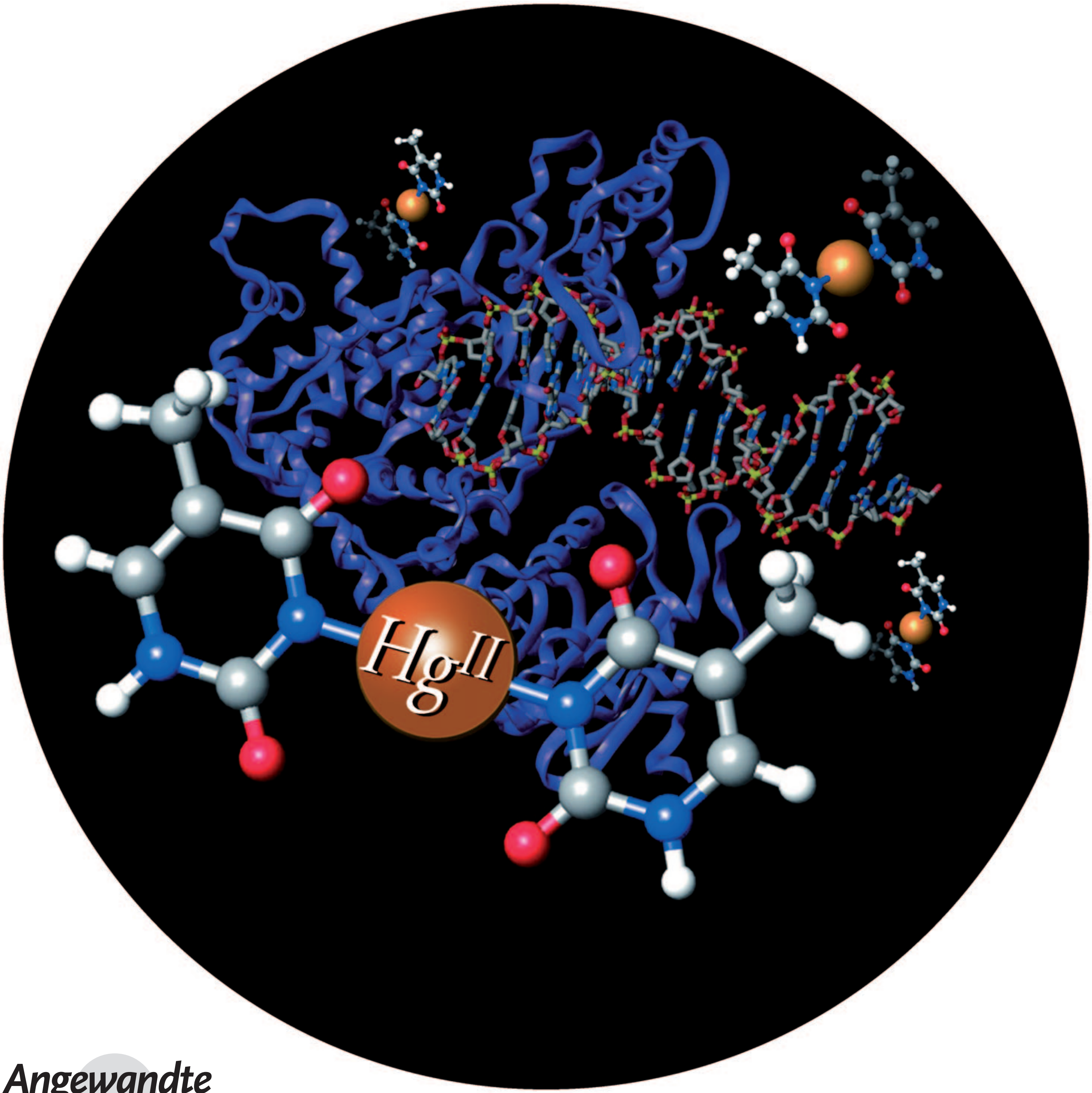
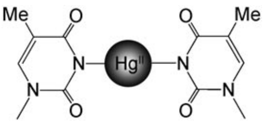


The Hg^{II} ion is a highly toxic heavy-metal ion that shows cytotoxic and mutagenic effects.[1,2] These effects of Hg^{II} ions have been explained by the modification of proteins,[3] such as replicative and repair enzymes, and by DNA single-strand breaks[1] arising from the formation of Hg^{II}–DNA complexes.[4] The binding mechanism of Hg^{II} ions with polynucleotides was studied initially by Katz, who proposed a mercury(II)-mediated thymine–thymine (T–T) pair (or "T–Hg^{II}–T" hereafter) induced by strand slippage, with Hg^{II} coordinated to both N3 positions of two thymine residues.[5] However, NMR spectroscopic studies of oligodeoxynucleotide (ODN)–Hg^{II} complexes revealed that Hg^{II} coordinated to the N1 position of the adenine residue and the O4 position of the thymine residue to form an A–Hg^{II}–T pair.[6] Recently, Ono and co-workers reported that Hg^{II} ions bind highly selectively to T–T mismatched base pairs in ODN duplexes to increase the thermal stability of the duplexes.[7] Other metal ions known to interact with nucleic acids did not show any notable effects on the melting behavior of the duplexes. The coordination sites of Hg^{II} ions were directly proved to be the N3 positions of both thymine moieties by NMR spectroscopy with ^{15}N labeling (Scheme 1).[8] This remarkable specificity for the coordination of Hg^{II} ions to T–T mismatched base pairs was applied to mercury(II)-ion sensing based on Förster resonance energy transfer.[9]



Scheme 1. Mercury(II)-mediated T–T mismatched base pair.

Another concern is the significance and role of Hg^{II} coordination to T–T mismatched base pairs in biological systems. It has been considered that the number and strength of hydrogen bonds in a base pair determine the efficiency and fidelity of DNA polymerases. Studies on artificial nucleobases that form base pairs with nonstandard Watson–Crick hydrogen bonding and non-hydrogen-bonding shape complementarity,[10,11] such as isoguanine–isocytosine, dκ–dX, and dZ–dF pairs, have shown that DNA polymerase can recognize these types of modified substrates and catalyze a replication reaction through the formation of the artificial base pairs. Such metal-ion-mediated base pairs as the T–Hg^{II}–T base pair are an alternative type of artificial base pair.[12] Herein, we report a primer-extension reaction in the presence of Hg^{II} ions and the misincorporation of thymidine 5′-triphosphate (TTP) into the site opposite T in a template strand by DNA polymerases to form the T–Hg^{II}–T base pair.

The Klenow fragment (KF) was used as a DNA polymerase in conjunction with the primed template, which has a nine-base single-strand region containing one T residue (Figure 1 a). To simplify PAGE analysis, a nine-base single-strand region containing no deoxyadenosine residues was used so that the extension reaction would basically afford only two products (a 19-mer and the full-length 24-mer) in the presence and absence of TTP and/or Hg^{II} ions. In preliminary experiments, we tested the effect of the concentration of dithiothreitol (DTT) on the reactions. Since we found that DTT at concentrations of 8–100 μM hardly affected the usual primer-extension reactions (see Figure S1 in the Supporting Information), we carried out extension reactions in the presence of DTT at a concentration of 8 μM to minimize the capture of Hg^{II} ions by the thiol groups of DTT.

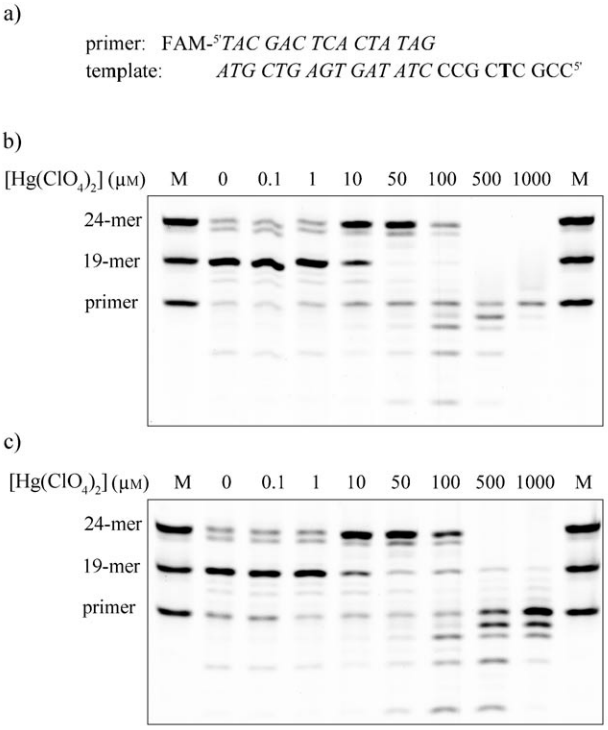


Figure 1. a) Sequences of the template and primer strands. The primer was fluorescence-labeled with fluorescein amidite (FAM) at the 5′ end. b) Primer-extension reactions by the Klenow fragment at various mercury(II)-ion concentrations (0–1000 μM). c) The same reactions as in (b) followed by treatment with DTT (2.5 mM) before PAGE analysis. The reaction mixtures (100 nM primer, 150 nM template, 10 μM TTP, 10 μM dGTP, 10 μM dCTP, 50 mM NaCl, 10 mM $MgCl_2$, 8 μM DTT, 10 mM Tris–HCl, pH 7.9; Tris = 2-amino-2-hydroxymethylpropane-1,3-diol) were incubated at 37 °C for 1 h. M indicates markers for the primer, 19-mer, and 24-mer.

First, we evaluated primer extensions at various mercury(II)-ion concentrations in the presence of TTP, 2′-deoxyguanosine 5′-triphosphate (dGTP), and 2′-deoxycytidine 5′-triphosphate (dCTP). Reactions at higher mercury(II)-ion concentrations led to weaker bands on the PAGE gels used for analysis (Figure 1 b). This phenomenon is probably due to the aggregation of DNA by the Hg^{II} ions at high concen-

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trations.[4a] Indeed, the effect was completely reversed by treatment with DTT (2.5 mM) before PAGE (Figure 1 c), in agreement with previous findings that the aggregation of DNA was completely reversed by complexing agents for Hg^{II} ions, such as chloride and cyanide ions.[4] In the absence of Hg^{II} ions, the reaction was terminated at the site opposite T in the template to afford the 19-mer product. At mercury(II)-ion concentrations of 10–50 μM, the full-length 24-mer was produced as the major product. However, at higher mercury(II)-ion concentrations (> 100 μM), no elongation products were formed, and substantial degradation of the primer was observed, possibly owing to inhibition of the polymerase activity and to the remaining $3' \rightarrow 5'$ exonuclease activity of KF. At a mercury(II)-ion concentration of 1000 μM, $3' \rightarrow 5'$ exonuclease activity was also partially inhibited.

Within the mercury(II)-ion concentration range of 10–100 μM, KF extended the primer to the full-length 24-mer in the absence of deoxyadenosine triphosphate (dATP). To establish the factors required for this extension reaction, we used various combinations of deoxynucleoside 5′-triphosphates (dNTPs) in the absence or presence of Hg^{II} ions. In the presence of dATP in addition to dGTP and dCTP, the extension reactions proceeded to afford the full-length 24-mer regardless of the presence or absence of Hg^{II} ions (15 μM; Figure 2, lanes 1 and 2). Thus, Hg^{II} ions at this concentration did not have a notable influence on the normal extension reaction. In the absence of dATP and TTP, the reaction was terminated at the site opposite T in the template to afford the 19-mer product regardless of the presence or absence of Hg^{II} ions (Figure 2, lanes 5 and 6). However, in the presence of TTP, the addition of Hg^{II} ions (15 μM) promoted the further elongation of the 19-mer product to afford the full-length 24-mer (Figure 2, lane 4). This reaction did not proceed in the absence of Hg^{II} ions (Figure 2, lane 3). These results indicate that this unusual primer extension proceeds only in the presence of both TTP and Hg^{II} ions and therefore that TTP is incorporated into the site opposite T in the template through coordination to Hg^{II} ions. The effects of the mercury(II)-ion concentration on the extension reaction for each dNTP combination are shown in Figure S2 of the Supporting Information.

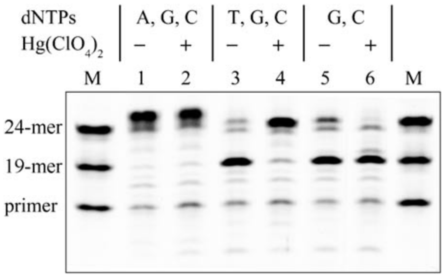

Figure 2. Primer-extension reactions by the Klenow fragment in the absence and presence of Hg^{II} ions (15 μM). The reaction mixtures (100 nM primer, 150 nM template, 10 μM dNTPs, 50 mM NaCl, 10 mM $MgCl_2$, 8 μM DTT, 10 mM Tris–HCl, pH 7.9) were incubated at 37 °C for 1 h. M indicates markers for the primer, 19-mer, and 24-mer.

In UV melting experiments, the stabilization of duplexes containing T–T mismatched base pairs by metal ions was reported to be highly selective for Hg^{II} ions over other DNA-binding metal cations.[7] The addition of Mn^{II}, Fe^{II}, Fe^{III}, Co^{II}, Cu^{II}, Zn^{II}, Pb^{II}, Ni^{II}, or Au^{I} to the primer-extension reaction did not result in the formation of the full-length 24-mer (Figure 3). Only Hg^{II} ions promoted the extension reaction to give the full-length 24-mer, in analogy to the selectivity for Hg^{II} ions in the stabilization of duplexes containing T–T mismatched base pairs.[7] To exclude the effects of the counteranion (perchlorate anion), reactions were carried out in the presence of $Hg^{II}(OAc)_2$ or $Mg^{II}(ClO_4)_2$. We found that $Hg^{II}(OAc)_2$ promoted the reaction, whereas $Mg^{II}(ClO_4)_2$ did not (see Figure S3 in the Supporting Information). Thus, Hg^{II} ions mediate TTP incorporation into the site opposite T in the template by KF. Furthermore, this phenomenon is not specific to KF: KOD Dash and Taq DNA polymerases also catalyzed this reaction (Figure 4).

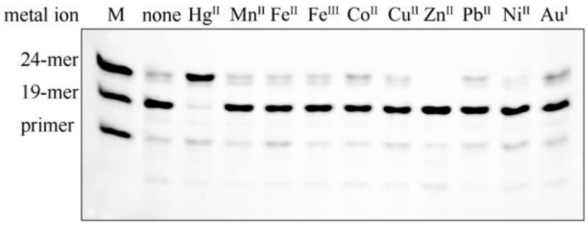

Figure 3. Primer-extension reactions by the Klenow fragment in the presence of a variety of metal ions (15 μM). The reaction mixtures (100 nM primer, 150 nM template, 10 μM TTP, 10 μM dGTP, 10 μM dCTP, 50 mM NaCl, 10 mM $MgCl_2$, 8 μM DTT, 10 mM Tris–HCl, pH 7.9) were incubated at 37 °C for 1 h. M indicates markers for the primer, 19-mer, and 24-mer.

KF was reported to have an active site that is 0.5–0.7 Å larger than natural Watson–Crick base pairs.[13] The enzyme

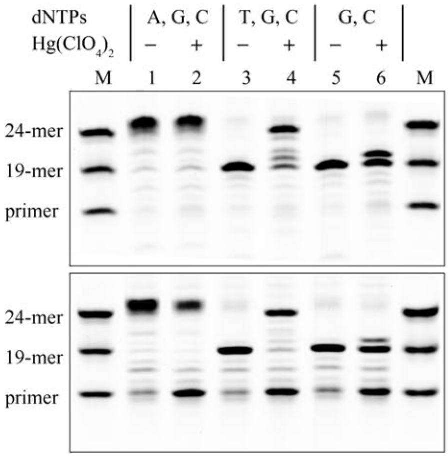

Figure 4. Primer-extension reactions by KOD Dash (top: enzyme (0.4 U), $Hg(ClO_4)_2$ (25 μM), 2 h; other reaction conditions as for Figure 2) and Taq polymerase (bottom: enzyme (0.8 U), $Hg(ClO_4)_2$ (45 μM), dNTPs (30 μM), 2 h; other reaction conditions as for Figure 2).

effectively incorporates a natural or modified dNTP to form a base pair that can be accommodated within the active site. In the replication process by KF, non-natural base pairs containing base-pair mismatches or a modified base (or bases) often prevent formation of the next base pair.[14] Thus, the geometry of a newly synthesized base pair is thought to be important for the next dNTP-incorporation step. In this study, KF selectively incorporated TTP into the site opposite T in the template in the presence of Hg^{II} ions; the enzyme then incorporated the next dNTPs to yield the full-length product. These results demonstrate that the T–Hg^{II}–T base pair has a similar size and geometry to those of natural Watson–Crick base pairs and is well accommodated within the active site of the enzyme.

In conclusion, it has been shown that DNA polymerases can utilize artificial nucleobases that form base pairs with non-natural hydrogen bonding and shape complementarity. We demonstrated herein that TTP was incorporated into the primer strand through the formation of a T–Hg^{II}–T base pair by DNA polymerases and that the enzymes recognized the metal-coordinated type of base pair and elongated the primer. Our findings clarify the effects of Hg^{II} ions on the functions of DNA polymerases and also suggest a potential mechanism for the mutagenic activity of Hg^{II} ions. Moreover, these results open new possibilities for the metal-ion-mediated enzymatic incorporation of a variety of artificial bases into oligonucleotides to expand the genetic alphabet,[10,11] and for the functional switching of ODNs through modification involving metal ions.[15]

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Keywords: DNA–metal-ion interactions · DNA polymerases · DNA structures · mercury · mismatched base pairs

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