DNA Technology

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"Illusionary" Polymerase Activity Triggered by Metal Ions: Use for Molecular Logic-Gate Operations**

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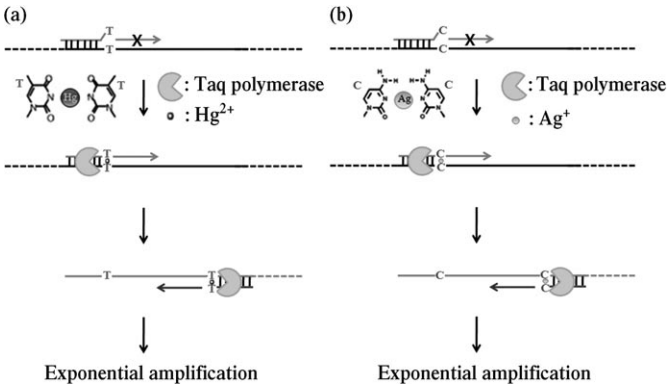
In recent years, an intense interest has grown in the interactions of nucleic acids with metal ions.[1] Examples of such novel interactions include the specific binding of aptamers with metal ions[2] and selective incorporation of metal ions as cofactors to promote the catalytic activities of nucleic acid enzymes (deoxyribozymes or ribozymes).[3] Furthermore, certain metal ions, such as Hg^{2+}, Ag^{+}, Cu^{2+}, Ni^{2+}, and Co^{2+}, specifically bind to nucleosides or ligandosides to form metal-ion-mediated base pairs. This nonnatural base paring is stabilized by coordination of the metal ions to the nucleosides in a manner that is different from natural hydrogen bonding between complementary nucleosides.[4]

The novel interaction of nucleic acids with metal ions has recently been utilized for the construction of molecular-scale logic gates, which are essential for the development of molecular-scale computers and other computational devices.[5] Most representatively, deoxyribozymes have been employed to build logic gates based on the fact that their catalytic activities can be regulated by the presence of specific metal ions.[6] However, these kinds of logic gates typically rely on relatively complicated designs for gate switching and frequently require the involvement of RNA or chimeric DNA as an operational substrate.[7] In addition to leading to high construction costs, this phenomenon ultimately requires complex operational features for controlling the system as a result of the susceptibility of RNA molecules to degradation.

From this perspective, it would be highly desirable to develop molecular-scale logic gates that operate in a more simple and cost-effective manner. The results of the investigation described below have led to a new, simple strategy for construction of molecular-scale logic gates that are based on the "illusionary" polymerase activity at the mismatched site triggered by metal ions. As illustrated in Scheme 1, the underlying principle for operation of this system relies on specific interactions between metal ions (Hg^{2+} or Ag^{+}) and the respective mismatched base pairs (thymine–thymine (T–T)[4a] or cytosine–cytosine (C–C)[4b]). Forward (F) and reverse



Scheme 1. Illustration of polymerase activity triggered by metal ions. a) Extension of T–T mismatched primer in the presence of Hg^{2+} ions. b) Extension of C–C mismatched primer in the presence of Ag^{+} ions.

(R) primers were designed to form T–T (Scheme 1a) or C–C (Scheme 1b) mismatches with template DNA at its 3′ end. The mismatched primers cannot be extended in the absence of the respective metal ions, because the terminal mismatching stalls action of the polymerase enzyme at the 3′ end, thus preventing the elongation reaction promoted by the polymerase.[8] However, in the presence of Hg^{2+} or Ag^{+} ions, the terminal T or C base at the 3′ end of the primer can form an nonnatural but stable T-Hg^{2+}-T or C-Ag^{+}-C base pair with template DNA. This stabilization induces the polymerase activity and, as a consequence, amplification products are formed (Scheme 1). This activity is termed illusionary polymerase activity herein because it is derived from the illusion of DNA polymerase that the metal-ion-mediated base pair is perfectly matched.

Figure 1 shows gel electrophoresis images of the products obtained from PCR mixtures containing F/R primers with terminal mismatched T (Figure 1a) or C base (Figure 1b) at the 3′ end. As envisioned, use of the T–T and C–C mismatched primers results in formation of gel bands that correspond to amplification products only when the respective Hg^{2+} and Ag^{+} ions are present (lane 3 in Figure 1a,b). In contrast, employment of perfectly matched primers results in the generation of amplification products regardless of whether or not the respective metal ions are present (lane 1, 4 in Figure 1a,b). Importantly, the fact that no significant difference is seen between the band intensities for systems with and without the metal ions indicates that the metal ions do not have an adverse effect on the polymerase reaction. To further support the proposal that the metal ions induce polymerase activity, melting curve analyses were performed for the extension products obtained from the T–T and C–C

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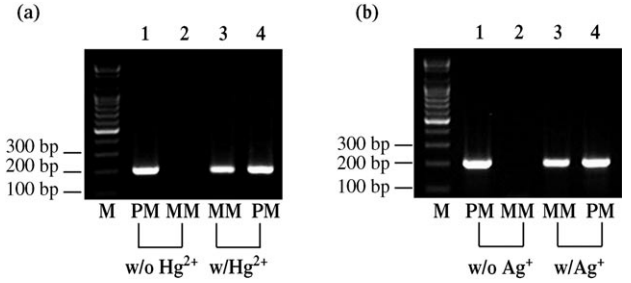

Figure 1. Gel electrophoresis images of the PCR products formed by polymerase activity triggered by metal ions. a) Extension of A–T perfectly matched primer (lane 1, 4) and T–T mismatched primer (lane 2, 3) in the absence or presence of Hg^{2+} ions. b) Extension of G–C perfectly matched primer (lane 1, 4) and C–C mismatched primer (lane 2, 3) in the absence or presence of Ag^{+} ions. Lane M indicates 100 base pair (bp) ladder size markers. PM: Perfectly matched primers. MM: Mismatched primers.

mismatched primers (Figure S1 in the Supporting Information). The mismatched primers show melting peaks for the extension products only when the respective metal ions (Hg^{2+} or Ag^{+}) are present. In contrast, melting peaks only for the hybridized primers are observed when metal ions are absent. Together, these results clearly demonstrate that polymerase activity is triggered by the presence of the specific metal ions.

To evaluate the specificity of metal ions in the induction of polymerase activity from the T–T or C–C mismatched primers, 13 different metal ions (Zn^{2+}, Ca^{2+}, Pb^{2+}, Mn^{2+}, Fe^{3+}, Cu^{2+}, K^{+}, Ni^{2+}, Co^{2+}, Mg^{2+}, Cd^{2+}, Hg^{2+}, and Ag^{+}; each 10 μM) were tested for their ability to promote amplification reactions. The results show that only Hg^{2+} and Ag^{+} ions cause the formation of PCR products from T–T (Figure 2a) and C–

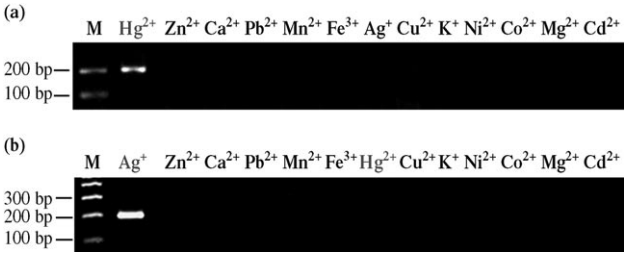

Figure 2. Selectivity of specific metal ions to trigger the polymerase activity. a) Extension of T–T mismatched primer in the presence of different metal ions. b) Extension of C–C mismatched primer in the presence of different metal ions. Lane M indicates 100 bp ladder size markers.

C (Figure 2b) mismatched primers, respectively. The fact that no detectable gel bands were observed when any of the other metal ions was added indicates an exceptionally high level of specificity for coordination of Hg^{2+} or Ag^{+} ions to the corresponding mismatched base pair.

The observations described above suggest that the "illusionary" activity of the polymerase induced by specific metal ions could be utilized for the identification of metal ions. Moreover, this new phenomenon could serve as the basis for novel molecular-scale logic gates through simple incorporation of a single mismatched base at the 3′ end of the primer. The logic gates would operate by using Hg^{2+} and Ag^{+} ions as inputs and DNA amplification as outputs. The output would be reported through either the appearance of an electrophoretic band or the fluorescence enhancement of EvaGreen fluorescent dyes specific to double-stranded (ds) DNA.

To explore this proposal, a YES gate, the simplest logic gate with a single input, was constructed (Figure 3). The YES

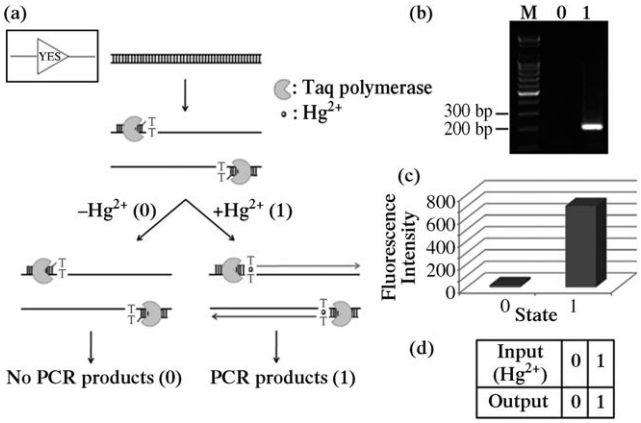

Figure 3. A YES logic gate system consisting of F/R primers with a single mismatched T base at the 3′ end by using Hg^{2+} ions as inputs. a) Illustration of the operational design of the YES gate. b) Gel electrophoresis analysis. (Lane M indicates 100 bp ladder size markers.) c) The fluorescence intensity at the 30th PCR cycle. d) Truth table. Input: (0) without Hg^{2+} ions, (1) with Hg^{2+} ions.

gate system faithfully follows the input (e.g., an output 0 from an input 0 and an output 1 from an input 1). In this system, Hg^{2+} ions, which form a stable T-Hg^{2+}-T complex, were used as inputs to trigger the polymerase activity from a T–T mismatched primer. The amplification products were formed in the presence of Hg^{2+} ions, while no product was generated in the absence of these ions, an observation that is in accord with proper execution of the YES logic gate (Figure 3). A PASS1 gate system that always gives an output of 1 regardless of inputs was then constructed by employing the same components found in the YES gate, but with pfu polymerase being used instead of Taq polymerase. Since pfu polymerase has proofreading 3′→5′ exonuclease activity, which Taq polymerase lacks, the primer can be extended even at the mismatched site after cleavage of the mismatched base at the 3′ end of the primer. As a consequence of this difference, PCR products are formed in systems either containing or not containing Hg^{2+} ions (Figure 4).

An AND gate, which gives an output of 1 only if both of the two inputs are held at 1, was constructed next. This gate is composed of F and R primers with a single mismatched T or C base, respectively, at the 3′ end and uses both Hg^{2+} and Ag^{+} ions as inputs. In this system, Hg^{2+} ions specifically interact with the mismatched T base pair at the 3′ end of the F primer, while Ag^{+} ions specifically coordinate with the mismatched C base pair at the 3′ end of the R primer. Thus, when only one of the two metal ions is present as an input, only one primer (F or

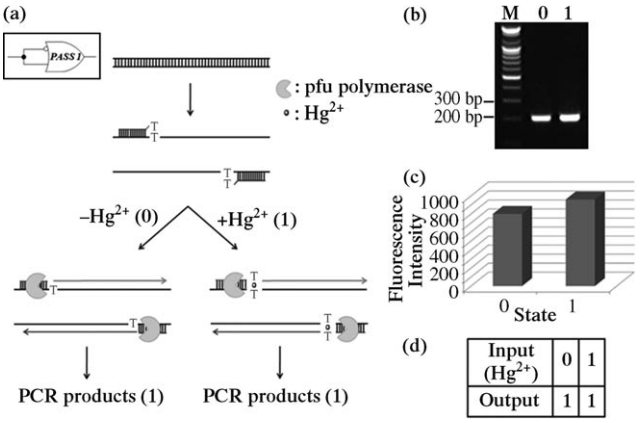

Figure 4. A PASS1 logic gate system consisting of F/R primers with a single mismatched T base at the 3′ end by using Hg^{2+} ions as inputs. a) Illustration of the operational design of the PASS1 gate. b) Gel electrophoresis analysis. c) Fluorescence intensity at the 30th PCR cycle. d) Truth table. Input: (0) without Hg^{2+} ions, (1) with Hg^{2+} ions.

R) corresponding to the applied ion can be extended by action of the polymerase activity. This leads to simple linear amplification but not to the exponential amplification that occurs in the normal PCR reaction. Consequently, insufficient product is formed to generate a detectable band on the electrophoretic gel. As expected, addition of either Hg^{2+} or Ag^{+} ions does not generate output signals, while addition of both metal ions (Hg^{2+} and Ag^{+}) facilitates exponential PCR amplification that results in the formation of detectable quantities of amplification products (Figure 5).

Finally, an OR gate, which produces an output of 1 when at least one of the two inputs is 1, was constructed. Figure 6a shows an outline of the design of the OR gate, which is comprised of two sets of F/R primers in which one functions

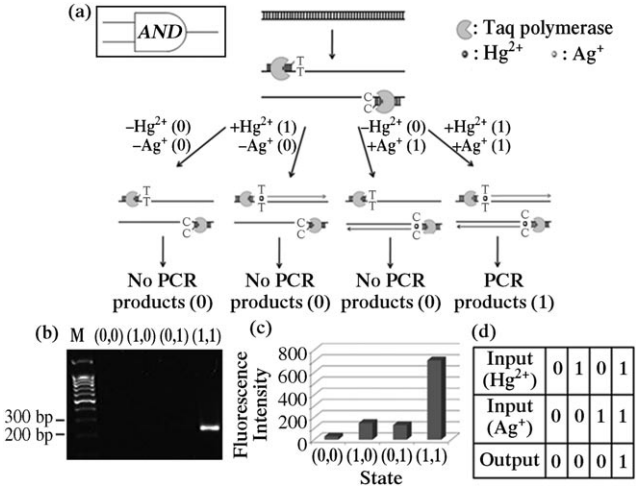

Figure 5. An AND logic gate system consisting of F/R primers with respective single mismatched T and C base at the 3′ end by using both Hg^{2+} and Ag^{+} ions as inputs. a) Illustration of the operational design of AND gate. b) Gel electrophoresis analysis. c) The fluorescence intensity at the 30th PCR cycle. d) Truth table. State: (0,0) without Hg^{2+} ions, without Ag^{+} ions; (1,0) with Hg^{2+} ions, without Ag^{+} ions; (0,1) without Hg^{2+} ions, with Ag^{+} ions; (1,1) with Hg^{2+} ions, with Ag^{+} ions.

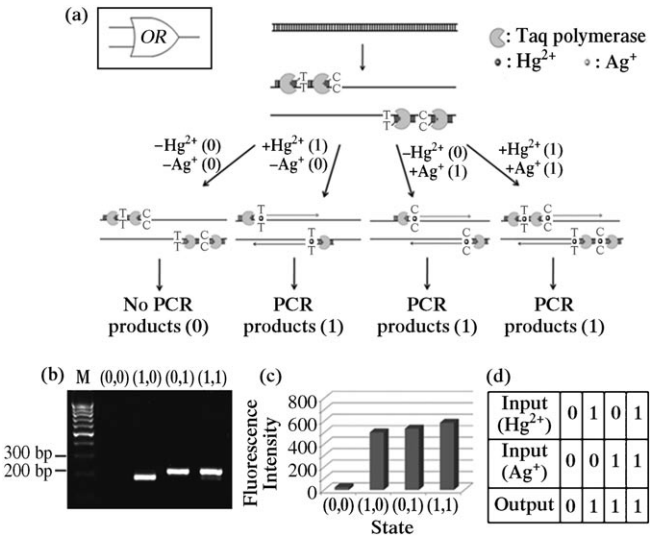

Figure 6. An OR logic gate system consisting of two sets of F/R primers, one with a single mismatched T base at the 3′end and the other with a single mismatched C base at the 3′ end by using both Hg^{2+} and Ag^{+} ions as inputs. a) Illustration of the operational design of OR gate. b) Gel electrophoresis analysis. c) The fluorescence intensity at the 30th PCR cycle. d) Truth table. State: (0,0) without Hg^{2+} ions, without Ag^{+} ions; (1,0) with Hg^{2+} ions, without Ag^{+} ions; (0,1) without Hg^{2+} ions, with Ag^{+} ions; (1,1) with Hg^{2+} ions, with Ag^{+} ions.

to form a T-Hg^{2+}-T base pair with the template in the presence of Hg^{2+} ions and the other to form a C-Ag^{+}-C base pair with the template in the presence of Ag^{+} ions. Since the Hg^{2+} or Ag^{+} ions interact independently with the corresponding T–T or C–C mismatched base pairs at the 3′ end of the primer, PCR amplification should take place in the presence of either one or both of the two metal ions. Electrophoretic or fluorescence output signals are indeed detected for this gate (Figure 6b,c).

The study described above has led to the discovery that metal ions (Hg^{2+} or Ag^{+}) that specifically interact with mismatched base pairs (T–T or C–C) can be employed to intentionally trigger polymerase activity. By utilizing this concept, we have successfully constructed a molecular-scale logic-gate system that uses Hg^{2+} or Ag^{+} ions as inputs and DNA amplification as an output. To our knowledge, this is the first time that key logic gates, which use PCR amplification as an output and metal ions as triggers, have been described. The most notable feature of the logic gates developed in this effort is their simplicity and cost-effective design. The only requirement for construction of the new logic gates is the incorporation of a single mismatched base (T or C) at the 3′ end of the primer and the application of metal ions (Hg^{2+} or Ag^{+}). We believe that this novel concept might be applicable to strategies for the design of a molecular translator, which would generate functional nucleic acids, such as aptamers, and deoxyribozymes by utilizing the unusual polymerase extension reaction and metal ions.

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