DNA Logic Gates

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Abstract: A conceptually new logic gate based on DNA has been devised. Methoxybenzodeazaadenine (MDA), an artificial nucleobase which we recently developed for efficient hole transport through DNA, formed stable base pairs with T and C. However, a reasonable hole-transport efficiency was observed in the reaction for the duplex containing an MDA/T base pair, whereas the hole transport was strongly suppressed in the reaction using a duplex where the base opposite MDA was replaced by C. The influence of complementary pyrimidines on the efficiency of hole transport through MDA was quite contrary to the selectivity observed for hole transport through G. The orthogonality of the modulation of these hole-transport properties by complementary pyrimidine bases is promising for the design of a new molecular logic gate. The logic gate system was executed by hole transport through short DNA duplexes, which consisted of the “logic gate strand”, containing hole-transporting nucleobases, and the “input strand”, containing pyrimidines which modulate the hole-transport efficiency of logic bases. A logic gate strand containing multiple MDA bases in series provided the basis for a sharp AND logic action. On the other hand, for OR logic and combinational logic, conversion of Boolean expressions to standard sum-of-product (SOP) expressions was indispensable. Three logic gate strands were designed for OR logic according to each product term in the standard SOP expression of OR logic. The hole-transport efficiency observed for the mixed sample of logic gate strands exhibited an OR logic behavior. This approach is generally applicable to the design of other complicated combinational logic circuits such as the full-adder.

Introduction

The design and construction of molecular systems that respond to chemical and/or photonic inputs by generating output signals, which are in accordance with logic gate behavior, has attracted considerable attention.1 Only in the past few years have scientists realized key experimental demonstrations of molecules that serve as molecular logic gates,2,3 as represented by rotaxane systems2 and photoinduced electron transfer (PET)-based sensors.4 However, despite broadly based and encouraging recent progress, a number of technical challenges remain to be overcome to make an easily designable, robust, and universal logic circuit integrated on the molecular scale. If a molecular system in which logic gates are integrated is successful, then molecular circuits should be within reach.

For a new generation of molecular logic gates, we focused on DNA, a biomolecule possessing well-regulated structures and the ability to store genetic information. DNA can form a self-assembled monolayer on gold surfaces,5 can form a complicated geometric structure,6 and can be site-specifically modified by chemical reactions.7 In the past decade, biochemical methods based on DNA hybridization and enzymatic treatment have been successfully employed for solving hard computational problems.8 A more valuable feature of DNA is that a long-range hole transport through DNA is readily accessible. A number of mechanistic and physical studies on DNA hole transport have suggested that DNA-based molecular electronics could be within reach.

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been reported. DNA hole transport is mediated by an extended π-stack and can promote oxidative damage to guanine bases from a remote site. A hole-transporting DNA base pair has MD A or G base for an X base, was hybridized with the strand containing MD A. This logic gate was designed to observe the output signal when hole transport occurred. The advantages of DNA logic gates are not only that the differences in hole-transport efficiencies between MD A/T and MD A/C base pairs is expected to constitute well-regulated bionanomaterials. We recently developed an artificial nucleobase, methoxybenzodeazaadenine (MD A), for efficient hole transport through DNA (Figure 1a). An MD A/T base pair behaved as a good mediator for hole transport and was not oxidatively decomposed. By combining this hole-transporting ability of MD A/T base pair with that of a G/C base pair, the design of molecular logic gates may become possible.

Here, we report on conceptually new molecular logic gates utilizing the hole-transport ability of short synthetic DNAs containing MD A. This logic gate was designed to observe the output signal when hole transport occurred. The advantages of DNA logic gates are not only that the differences in hole-transport efficiencies between MD A/T and MD A/C base pairs is used but also that all types of logic gates can be easily designed according to a simple protocol. Thus, we show a general protocol for the preparation of DNA logic gates and circuits.

**Experimental Section**

**DNA Synthesis and Characterization.** DNAs used for this study were synthesized by the conventional phosphoramidite method by using
Figure 3. Schematic illustration of a DNA logic gate system. The “logic gate strand” (green line), which has “logic bases” (X<sub>n</sub>), such as MD A and G, was hybridized with the “input strand” (orange line), which contains “input pyrimidines” (Y<sub>b</sub>) opposite logic bases. The duplex also includes a photosensitizer S for hole injection to DNA and two GGG sites for hole detection. Hole transport is triggered by photoirradiation with a transilluminator (312 nm) at 0 °C. The results of the hole transport are analyzed as described in Figure 2. The hole-transport efficiency of the logic gate strand as an output is defined by the ratio of oxidative strand cleavage (G<sub>c</sub>/G<sub>D</sub>). 

Preparation of 32 P-5'-End-Labeled Oligomers. The logic gate strand (DNA 400 pmol) was 5'-end-labeled by phosphorylation with 4 µL of [γ-32P]ATP (Amersham) and T4 polynucleotide kinase using a standard procedure. The 5'-end-labeled DNA was recovered by ethanol precipitation and further purified by 15% denaturing polyacrylamide gel electrophoresis (PAGE) and isolated by the crush and soak method.

Hole-Transport Experiment and PAGE Analysis. The logic gate strand (2.0 × 10<sup>5</sup> cpm) was hybridized with the input strand in 10 mM sodium cacodylate buffer (pH 7.0). Hybridization was achieved by heating the sample at 90 °C for 5 min and slowly cooling to room temperature. Photoirradiation was then carried out for a solution containing duplex (1 µM strand concentration) in 10 mM sodium cacodylate buffer at pH 7.0. The mixture was irradiated with a transilluminator (312 nm) at a distance of 3 cm at 0 °C for 45 min. After irradiation, all reaction mixtures were precipitated with the addition of 10 µL of 3 M sodium acetate, 20 µL of herring sperm DNA (50 µM base pair concentration), and 800 µL of ethanol. The

Results and Discussion

The synthesis of MD A-containing DNA was readily achieved from 4-chloro-6-methoxy-1H-pyrimido[4,5-b]indole according to the protocol reported earlier.10,11 The thermal stabilities of the MD A-containing duplexes were initially evaluated by determining the melting temperature ($T_m$) of the duplexes, S$^{-}$(CGCAATMDATAACGC)-3'/S$^{-}$(GGCTTAYATTGCG)-3' (Y = T, C, A, or G), from the change of $A_{260}$ on heating. The $T_m$ data are summarized in Table 1. The duplex containing an MD A/T base pair showed a $T_m$ value (54.6 °C) close to that of an A/T base pair (52.5 °C), suggesting that the duplex containing an MD A/T base pair is thermally stable. The most striking aspect of the result is that the stable base pair with MD A was observed for C. The stability observed in the duplex containing a "mismatched" A/C base pair (41.4 °C) remarkably decreased as compared with that for an A/T base pair, whereas the $T_m$ of the duplex containing an MD A/C base pair (52.0 °C) was close to that containing an MD A/T base pair. Furthermore, the influence of pH of the solution on the thermal stabilities of the duplexes containing an MD A/C base pair was examined. The stability of the duplex containing an MD A/C base pair was highest at pH 5–6 (Figure 1b). This behavior was similar to that of the duplex containing an A/C base pair. An A/C base pair is known to form a protonated wobble base pair at a low pH.12 Thus, an MD A/C base pair should also form a wobble base pair mediated by a proton or a water molecule as shown in Figure 1c.13 If so, the formation of the MD A/C base pair in duplex DNA would cause disruption of π-stacking and lowering of HOMO. Systematic π-stacking in duplex DNA14 and ionization potentials of hole-transporting bases15 are significantly important factors in hole transport in DNA. Therefore, the formation of an MD A/C base pair will be unfavorable for effective hole transport in DNA and lead to the suppression of hole transport.

Therefore, we evaluated the modulation of the hole-transport efficiency of MD A-containing DNA, which occurred by pyrimidines forming a base pair with MD A. The sequence and experimental results are shown in Figure 2a and 2b, respectively. The duplex contains the photosensitizer, cyanobenzophenone-substituted uridine (S), for hole injection to DNA,16 and two GGG sites for hole detection. GGG sites, which are known as an effective hole trap,10,11 were incorporated into both 5'- and 3'-sides of an MD A base. Hole transport was triggered by photoradiation with a transilluminator (312 nm) at 0 °C. The result of the hole transport was visualized as the oxidative strand cleavage at GGG sites by polyacrylamide gel electrophoresis (PAGE) analysis after hot piperidine treatment of reaction samples. The hole-transport efficiency of the logic gate strand was defined by the ratio of oxidative strand cleavage at the distal GGG (G$_b$) vs the proximal GGG (G$_a$). In the reaction for the duplex containing an MD A/T base pair, a reasonable hole-transport efficiency (damage ratio, G$_b$/G$_a$ = 0.39) was observed as reported earlier.10 On the other hand, in the reaction using a duplex where the base opposite MD A was replaced by C, the G$_b$/G$_a$ damage ratio was negligible (G$_b$/G$_a$ < 0.01), indicating that the hole transport was strongly suppressed. The influence of complementary pyrimidines on the efficiency of hole transport

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through MD A is quite contrary to the selectivity observed for hole transport through G; i.e., a base pair with C is a good hole carrier, but a base pair with T strongly suppresses hole transport (Figure 2c). The orthogonality of the modulation of these hole-transport properties by complementary pyrimidine bases will be promising for the design of a new molecular logic gate. In other words, if input signals “1” and “0” are applied to T and C, respectively, then the hole transport mediated by MD A would behave like a YES logic and that mediated by G would behave like a NOT logic that performs inversions.

Based on the results described above, we designed the outline of a DNA-based logic gate as shown in Figure 3. The “logic gate strand” (green), which contains hole-transporting nucleobases (logic bases), such as MD A and guanine (G), was hybridized with the “input strand” (yellow), which contains pyrimidines that modulate the hole-transport efficiency of logic bases (input pyrimidines). The duplex also contains the photosensitizer S and two GGG sites as the experiment described above. After a phototriggered hole-transport reaction, the hole-transport efficiency of the logic gate strand was defined by the damage ratio, \( G_b / G_a \).

Addition of extra base pairs modulating hole transport through DNA would be a rational approach to logic systems that handle two or more inputs. We have designed a logic gate strand containing two MD A bases in series (it denotes “MD A–MD A”) (Figure 4a). In this sequence, logic bases were separated by two T/A base pairs in order to work independently. Thus, the size of one input region, including spacers, was three base pairs; i.e., corresponding to 1 nm in length. We hybridized the logic gate strand with input strands containing different combinations of two input pyrimidines (YA–Y B ). When the input strand where both input pyrimidines were T was used, the strand cleavage at the Gb site as an output signal was observed (\( G_b / G_a < 0.01 \)), indicating that effective hole transport had occurred (Figure 4a). When the input pyrimidines Y1 and/or Y 2 were C, hole transport to the G b site was strongly suppressed (\( G_b / G_a < 0.01 \)). These results indicate that a logic gate strand containing multiple MD A bases in series can provide the basis for a sharp AND logic action.

Next, we designed an OR DNA logic gate, which is expressed by the sum of inputs. We initially converted the OR equation, “A + B”, to a standard sum-of-product (SOP) expression, in which all the variables in the domain appear in each product term. Conversion to standard SOP expressions remarkably facilitated the sequence design of DNA logic gates. We designed three logic gate strands for OR logic, MD A–MD A, MD A–G and G–MD A, according to each product term in the

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standard SOP expression of OR logic, and analyzed the hole-
transport reactions of a mixture of these strands hybridized with
an input strand in a single cuvette (Figure 4b). The resulting
PAGE analysis exhibited the strand cleavage bands at the $G_b$
site when any of the input pyrimidines was T ($G_b/G_a = 0.12-0.08$, respectively). Only for the C–C input, the strand cleavage
at the $G_b$ site was not observed ($G_b/G_a < 0.01$). This cleavage
pattern observed for the mixed sample exhibited an OR logic
behavior.

YES, NOT, AND, and OR gates are the basic logic gates
from which all logic functions are constructed; therefore, we
can easily create complicated logic gates such as NAND, NOR,
and XOR in a single cuvette using hole-transporting DNA
programmed according to a protocol, as explained below.
Boolean multiplication is expressed by arranging the logic bases
($M_D A$ and G as an inversion of $M_D A$) in a logic gate strand. The
number of variables in each product term of standard SOP
expressions is equivalent to the number of logic bases in the
logic gate strand. The Boolean addition of standard SOP
expressions is solved by simultaneously analyzing the reactions
of logic gate strands, of which the number is equivalent to the
number of the product terms in standard SOP expressions. DNA
logic gates designed according to this protocol would offer the
possibility of obtaining more complicated systems capable of
performing as an adder, one of the combinational logics. A full
adder, as the basic component of computational arithmetic in
semiconductor technology, is a digital circuit that adds three
inputs ($A$, $B$, and $C_{in}$) to produce two outputs ($S$ and $C_{out}$)
through AND, OR, and XOR operations. Based on the method
described above, we have created a full-adder logic. Figure 5a
shows a schematic representation of DNA logic gates that can
perform full-adder operations. We prepared two cuvettes for
two outputs, and logic gate strands designed according to the
standard SOP expression of full-adder logic were hybridized
with an input strand containing three input piperidines $Y_A-Y_B-Y_{cin}$ in each cuvette. The hole-transport efficiency obtained
by PAGE analysis after photolysis for each cuvette is
summarized in Figure 5b. The strand cleavage patterns observed
for each input strand were in very good agreement with data
shown in the truth table of the full adder (Figure 5c). This DNA
logic gate system designed according to our protocol satisfied
full-adder logic.

**Conclusion**

In conclusion, we have demonstrated the design and develop-
ment of a new DNA logic gate. We indicated three important
factors that are prerequisites for designing DNA logic gates:
(i) logic bases, $M_D A$ and G, are applied to a YES gate and a
NOT gate, respectively, because the hole-transport behaviors
of MDA and G modulated by complementary pyrimidines are
orthogonal to each other, (ii) logic gate strands, where logic
bases are arranged in series, act as an AND logic; and (iii)
conversion of Boolean expressions for OR logic and combina-
tional logic to standard SOP expressions facilitates the design
of DNA logic gates. This principle is easily applicable to the
design of complicated combinational logic circuits. Our DNA
logic gate systems, which respond to a given combination of
hole-transport-controllable base pairs, will open a way to the
further development encompassing well-regulated molecular
electronic devices and biosensors.