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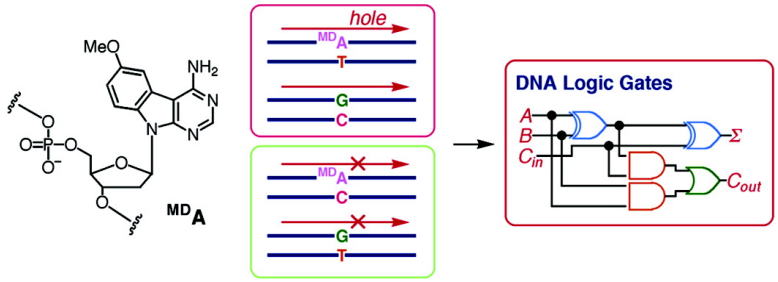

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DNA Logic Gates

Akimitsu Okamoto,*,†,§ Kazuo Tanaka,†,§ and Isao Saito*,‡,§

Contribution from the Department of Synthetic Chemistry and Biological Chemistry, Faculty of Engineering, Kyoto University, Kyoto 615-8510, Japan; NEWCAT Institute, Faculty of Engineering, Nihon University, Tamura, Koriyama 963-8642, Japan; and SORST, Japan Science and Technology Corporation, Kyoto 615-8510, Japan

Received April 24, 2004; E-mail: okamoto@sbchem.kyoto-u.ac.jp; saito@mech.ce.nihon-u.ac.jp

Abstract: A conceptually new logic gate based on DNA has been devised. Methoxybenzodeazaadenine (^{MD}A), 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 ^{MD}A/T base pair, whereas the hole transport was strongly suppressed in the reaction using a duplex where the base opposite ^{MD}A was replaced by C. The influence of complementary pyrimidines on the efficiency of hole transport through ^{MD}A 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 ^{MD}A 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,[1,2] as represented by rotaxane systems[3] 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

† Kyoto University.
‡ Nihon University.
§ SORST.

(1) (a) *Molecular Devices and Machines, A Journey into the Nanoworld*; Balzani, V., Venture, M., Credi, A., Eds.; Wiley-VCH: Weinheim, Germany, 2003. (b) *Molecular Switches*; Feringa, B., Ed.; Wiley-VCH: Weinheim, Germany, 2002.
(2) (a) de Silva, A. P.; McClenaghan, N. D. *Chem.—Eur. J.* **2003**, *10*, 574−586. (b) Raymo, F. M. *Adv. Mater.* **2002**, *14*, 401−414. (c) de Silva, A. P.; Gunatatne, H. N. Q.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515−1566. (d) de Silva, A. P.; Gunatatne, H. N. Q.; McCoy, C. P. *Nature* **1993**, *364*, 42−44.
(3) (a) Collier, C. P.; Wong, E. W.; Belohradsky, M.; Raymo, F. M.; Stoddart, J. F.; Kuekes, P. J.; Williams, R. S.; Heath, J. R. *Science* **1999**, *285*, 391−394. (b) Asakawa, M.; Ashton, P. R.; Balzani, V.; Credi, A.; Mattersteig, G.; Matthews, O. A.; Montalti, M.; Spencer, N.; Stoddart, J. F.; Venturi, M. *Chem.—Eur. J.* **1997**, *3*, 1992−1996. (c) Credi, A.; Balzani, V.; Langford, S. J.; Stoddart, J. F. *J. Am. Chem. Soc.* **1997**, *119*, 2679−2681.
(4) (a) Guo, X.; Zhang, D.; Wang, T.; Zhu, D. *Chem. Commun.* **2003**, 914−915. (b) De, S.; Pal, A.; Pal, T. *Langmuir* **2000**, *16*, 6855−6861. (c) de Silva, A. P.; Gunaratne, H. Q. N.; Maguire, G. E. M. *J. Chem. Soc., Chem. Commun.* **1994**, 1213−1214.
(5) (a) Nakamura, F.; Ito, E.; Sakao, Y.; Ueno, N.; Gatuna, I. N.; Ouchi, F. S.; Hara, M. *Nano Lett.* **2003**, *3*, 1083−1086. (b) Sortino, S.; Petralia, S.; Condorelli, G. G.; Conoci, S.; Condorelli, G. *Langmuir* **2003**, *19*, 536−539. (c) Huang, E.; Satjapipat, M.; Han, S.; Zhou, F. *Langmuir* **2001**, *17*, 1215−1224.
(6) (a) Seeman, N. C. *Nature* **2003**, *421*, 427−431. (b) Mao, C.; LaBean, T. H.; Reif, J. H.; Seeman, N. C. *Nature* **2000**, *407*, 493−496.
(7) (a) Goodchild, J. *Bioconjugate Chem.* **1990**, *1*, 165−186. (b) Uhlmann, E.; Peyman, A. *Chem. Rev.* **1990**, *90*, 543−590. (c) Verma, S.; Eckstein, F. *Annu. Rev. Biochem.* **1998**, *67*, 99−134. (d) Okamoto, A.; Tainaka, K.; Saito, I. *Tetrahedron Lett.* **2002**, *43*, 4581−4583. (e) Okamoto, A.; Taiji, T.; Tainaka, K.; Saito, I. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1895−1896. (f) Virta, P.; Katajisto, J.; Niittymaki, T.; Lonnberg, H. *Tetrahedron* **2003**, *59*, 5137−5174.

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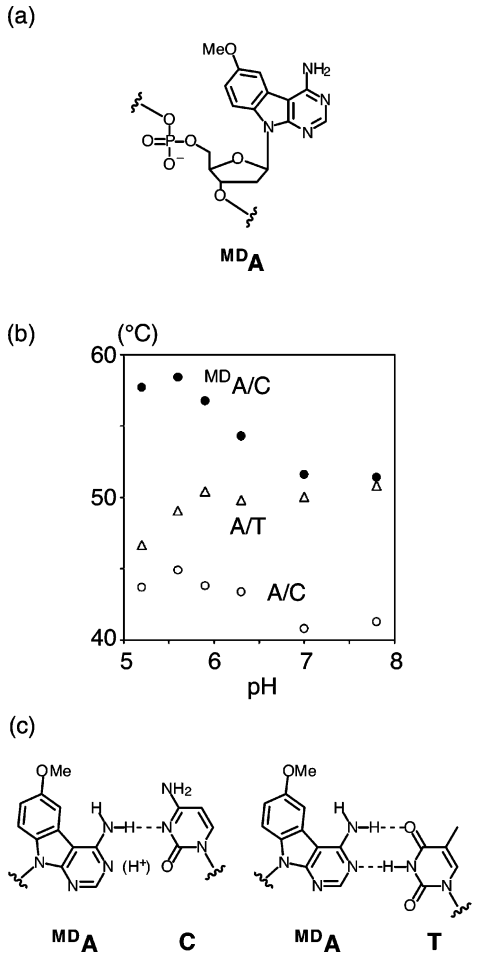


Figure 1. Thermal stability of the duplex containing an ^{MD}A/C base pair. (a) A hole-transporting nucleotide, ^{MD}A. (b) The plots of duplex-melting temperature (T_m) vs pH. T_m values for 5′-d(CGCAAT**X**TAACGC)-3′/5′-d(GCGTTA**Y**ATTGCG)-3′ duplexes (2.5 μM) were measured in a given pH of 50 mM sodium phosphate/0.1 M sodium chloride. **X**/**Y** = ^{MD}A/C (●), A/T (△), or A/C (○). (c) A proposed wobble ^{MD}A/C base pair and an ^{MD}A/T Watson−Crick base pair.

been reported.[9] DNA hole transport is mediated by an extended and well-defined π-stack and can promote oxidative damage to guanine bases from a remote site. A hole-transporting DNA is promising as a new nanoscale electronic device, which is expected to constitute well-regulated bionanomaterials. We recently developed an artificial nucleobase, methoxybenzodeazaadenine (^{MD}A), for efficient hole transport through DNA (Figure 1a).[10] An ^{MD}A/T base pair behaved as a good mediator

Table 1. Melting Temperatures (T_m) for 5′-d(CGCAAT**X**TAACGC)-3′/5′-D(GCGTTAYATTGCG)-3′ Duplexes[a]

		T_m (°C)			
X	Y =	T	C	A	G
^{MD}A		54.6	52.0	42.1	48.0
A		52.5	41.4	40.5	46.0

[a] Samples (2.5 μM duplex) were measured in 50 mM sodium phosphate/0.1 M sodium chloride (pH 7.0).

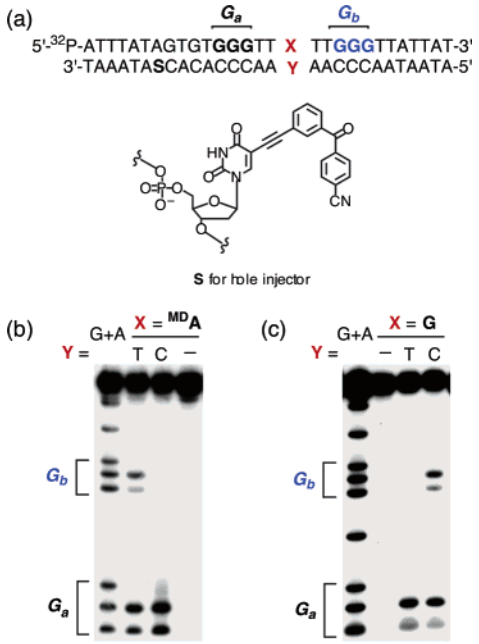


Figure 2. Efficiency of DNA hole transport through logic bases ^{MD}A and G. (a) DNA sequence used for this study. The ^{32}P-labeled strand, which has ^{MD}A or G base for an X base, was hybridized with the strand containing T or C base (Y) opposite an X base. The duplex also includes a photosensitizer, cyanobenzophenone-substituted uridine (S) for hole injection to DNA, and two GGG sites for hole detection. The duplexes in 10 mM sodium cacodylate (pH = 7.0) were photoirradiated (312 nm) at 0 °C for 45 min followed by a hot piperidine treatment (90 °C, 20 min). The result of the hole-transport reaction was visualized as the oxidative strand cleavage at the distal GGG (G_b) site (red) by polyacrylamide gel electrophoresis 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 G_b vs the proximal GGG (G_a). (b) Hole transport through ^{MD}A. (c) Hole transport through G. "G + A" lanes are Maxam−Gilbert G + A sequencing lanes. "−" lanes are control lanes (no hybridization).

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

(8) (a) Stojanovic, M. N.; Mitchell, T. E.; Stefanovic, D. *J. Am. Chem. Soc.* **2002**, *124*, 3555−3561. (b) Rothemund, P. W. K.; Adleman, L. *Science* **2002**, *296*, 499−502. (c) Braich, R. S.; Chelyapov, N.; Johnson, C.; Rothemund, P. W. K.; Adleman, L. *Science* **2002**, *296*, 499−502. (d) Benenson, Y.; Paz-Elizur, T.; Adar, R.; Keinan, E.; Livneh, Z.; Shapiro, E. *Nature* **2001**, *414*, 430−434. (e) Sakamoto, K.; Gouzu, H.; Komiya, K.; Kiga, D.; Yokoyama, S.; Yokomori, T.; Hagiya, M. *Science* **2000**, *288*, 1223−1226. (f) Liu, Q.; Wang, L. M.; Frutos, A. G.; Condon, A. E.; Corn, R. M.; Smith, L. M. *Nature* **2000**, *403*, 175−179. (g) Adleman, L. *Sci. Am.* **1998**, *279*, 54−61. (g) Gibbons, A.; Amos, M.; Hodgson, D. *Curr. Opin. Biotechnol.* **1997**, *8*, 103−196. (h) Adleman, L. *Science* **1994**, *266*, 1021−1024.

(9) (a) Hall, D. B.; Holmlin, R. E.; Barton, J. K. *Nature* **1996**, *382*, 731−735. (b) Schuster, G. B. *Acc. Chem. Res.* **2000**, *33*, 253−260. (c) Giese, B. *Acc. Chem. Res.* **2000**, *33*, 631−636. (d) Lewis, F. D.; Letsinger, R. L.; Wasielewski, M. R. *Acc. Chem. Res.* **2001**, *34*, 159−170.(e) Fink, H. W.; Schönenberger, C. *Nature* **1999**, *398*, 407−410. (f) Porath, D.; Bezryadin, A.; de Vries, S.; Dekker, C. *Nature* **2000**, *403*, 635−638. (g) Cai, L.; Tabata, H.; Kawai, T. *Appl. Phys. Lett.* **2000**, *77*, 3105−3106.

(10) Okamoto, A.; Tanaka, K.; Saito, I. *J. Am. Chem. Soc.* **2003**, *125*, 5066−5071.

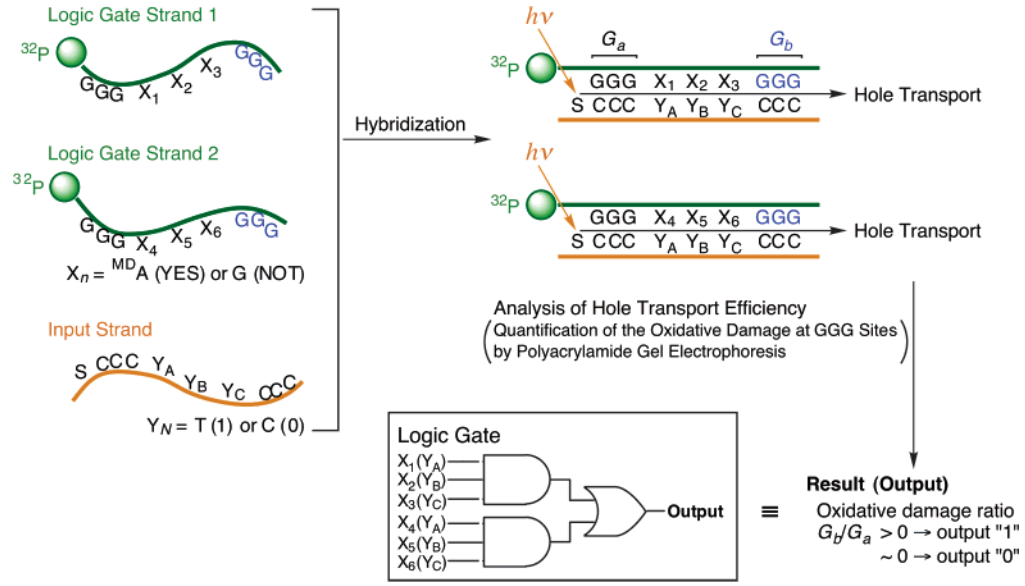


Figure 3. Schematic illustration of a DNA logic gate system. The "logic gate strand" (green line), which has "logic bases" (X_n), such as ^{MD}A and G, was hybridized with the "input strand" (orange line), which contains "input pyrimidines" (Y_N) opposite logic bases. The duplex also includes a photosensitizer 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_b/G_a).

an Applied Biosystems 392 DNA/RNA synthesizer. Phosphoroamidites were synthesized according to the protocol reported earlier.[10,11] Synthesized DNA was purified by reverse phase HPLC on a 5-ODS-H column (10 × 150 (W), elution with a solvent mixture of 0.1 M triethylammonium acetate (TEAA), pH 7.0, linear gradient over 30 min from 5% to 20% acetonitrile at a flow rate of 3.0 mL/min). An aliquot of purified DNA solution was fully digested with calf intestine alkaline phosphatase (50 U/mL), snake venom phosphodiesterase (0.15 U/mL), and P1 nuclease (50 U/mL) at 37 °C for 3 h. Digested solution was analyzed by HPLC on a Cosmosil 5C-18AR or CHEMCOBOND 5-ODS-H column (4.6 × 150 (W), elution with a solvent mixture of 0.1 M triethylammonium acetate (TEAA), pH 7.0, linear gradient over 20 min from 0% to 20% acetonitrile at a flow rate of 1.0 mL/min). Concentration of each DNA was determined by comparing a given peak area with those of 0.1 mM standard solution containing dA, dC, dG, and dT. Each DNA was characterized by MALDI-TOF MS; 5′-d(CGCAAT^{MD}ATAACGC)-3′, *m/z* 4005.66 (calcd for [M − H]$^-$ 4005.69); 5′-d(ATTTATAGTGTGGGTT^{MD}ATTGGGTTATTAT)-3′, *m/z* 9061.89 (calcd for [M − H]$^-$ 9059.34); 5′-d(ATTTATAGTGTGGGTT^{MD}ATT^{MD}ATTGGGTTATTAT)-3′, *m/z* 10092.2 (calcd for [M − H]$^-$ 10092.61); 5′-d(ATTTATAGTGTGGGTTGTT^{MD}ATTGGGTTATTAT)-3′, *m/z* 10029.5 (calcd for [M − H]$^-$ 10029.51); 5′-d(ATTTATAGTGTGGGTT^{MD}ATTGTTGGGTTATTAT)-3′, *m/z* 10030.5 (calcd for [M − H]$^-$ 10029.51); 5′-d(ATTTATAGTGTGGGTT^{MD}ATT^{MD}ATTGGGTTATTAT)-3′, *m/z* 11093.8 (calcd for [M − H]$^-$ 11093.30); 5′-d(ATTTATAGTGTGGGTT^{MD}ATTGTTGTTGGGTTATTAT)-3′, *m/z* 10968.2 (calcd for [M − H]$^-$ 10967.11); 5′-d(ATTTATAGTGTGGGTTGTT^{MD}ATTGTTGGGTTATTAT)-3′, *m/z* 10968.2 (calcd for [M − H]$^-$ 10967.11); 5′-d(ATTTATAGTGTGGGTTGTTGTT^{MD}ATTGGGTTATTAT)-3′, *m/z* 10967.9 (calcd for [M − H]$^-$ 10967.11); 5′-d(ATTTATAGTGTGGGTTGTT^{MD}ATT^{MD}ATTGGGTTATTAT)-3′, *m/z* 11030.7 (calcd for [M − H]$^-$ 11030.20); 5′-d(ATTTATAGTGTGGGTT^{MD}ATTGTT^{MD}ATTGGGTTATTAT)-3′, *m/z* 11031.6 (calcd for [M − H]$^-$ 11030.20); 5′-d(ATTTATAGTGTGGGTT^{MD}ATT^{MD}ATTGTTGGGTTATTAT)-3′, *m/z* 11031.7 (calcd for [M − H]$^-$ 11030.20); 5′-d(ATAATAACCCAATAACCCACACSATAAAT)-3′, *m/z* 8988.59 (calcd for [M − H]$^-$ 8988.96); 5′-d(ATAATAACCCAACAACCCACACSATAAAT)-3′, *m/z* 8973.27 (calcd for [M − H]$^-$ 8973.95); 5′-d(ATAATAACCCAATAATAACCCACACSATAAAT)-3′, *m/z* 9918.98 (calcd for [M − H]$^-$ 9919.56); 5′-d(ATAATAACCCAACAATAACCCACACSATAAAT)-3′, *m/z* 9905.74 (calcd for [M − H]$^-$ 9904.55); 5′-d(ATAATAACCCAATAACAACCCACACSATAAAT)-3′, *m/z* 9905.10 (calcd for [M − H]$^-$ 9904.55); 5′-d(ATAATAACCCAACAACAACCCACACSATAAAT)-3′, *m/z* 9889.58 (calcd for [M − H]$^-$ 9889.53); 5′-d(ATAATAACCCAACAACAACAACCCACACSATAAAT)-3′, *m/z* 10805.6 (calcd for [M − H]$^-$ 10805.14); 5′-d(ATAATAACCCAACAATAACAACCCACACSATAAAT)-3′, *m/z* 10820.6 (calcd for [M − H]$^-$ 10820.15); 5′-d(ATAATAACCCAACAACAATAACCCACACSATAAAT)-3′, *m/z* 10819.5 (calcd for [M − H]$^-$ 10820.15); 5′-d(ATAATAACCCAACAATAATAACCCACACSATAAAT)-3′, *m/z* 10835.3 (calcd for [M − H]$^-$ 10835.16); 5′-d(ATAATAACCCAATAACAACAACCCACACSATAAAT)-3′, *m/z* 10821.4 (calcd for [M − H]$^-$ 10820.15); 5′-d(ATAATAACCCAATAATAACAACCCACACSATAAAT)-3′, *m/z* 10836.6 (calcd for [M − H]$^-$ 10835.16); 5′-d(ATAATAACCCAATAACAATAACCCACACSATAAAT)-3′, *m/z* 10835.5 (calcd for [M − H]$^-$ 10835.16); 5′-d(ATAATAACCCAATAATAATAACCCACACSATAAAT)-3′, *m/z* 10850.7 (calcd for [M − H]$^-$ 10850.17).

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-^{32}P]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 DNA (2.0 × 10^5 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

(11) (a) Showalter, H. D. H.; Bridges, A. J.; Zhou, H.; Sercel, A. D.; McMichael, A.; Fry, D. W. *J. Med. Chem.* **1999**, *42*, 5464−5474. (b) Okamoto, A.; Tanaka, K.; Fukuta, T.; Saito, I. *J. Am. Chem. Soc.* **2003**, *125*, 9296−9297.

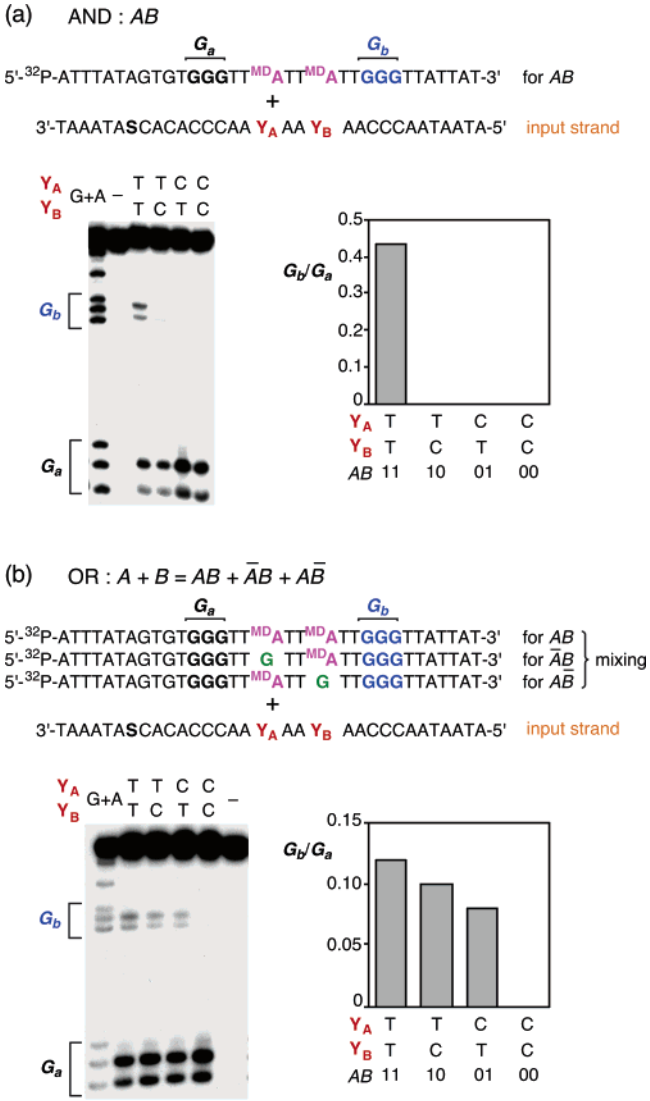


Figure 4. (a) AND operation and (b) OR operation by hole transport through designed DNA duplexes. The duplexes in 10 mM sodium cacodylate (pH = 7.0) were photoirradiated (312 nm) at 0 °C for 45 min followed by a hot piperidine treatment (90 °C, 20 min). The results of the hole transport were analyzed as described in Figure 2. "G + A" lanes are Maxam–Gilbert G + A sequencing lanes. "–" lanes are control lanes (no hybridization).

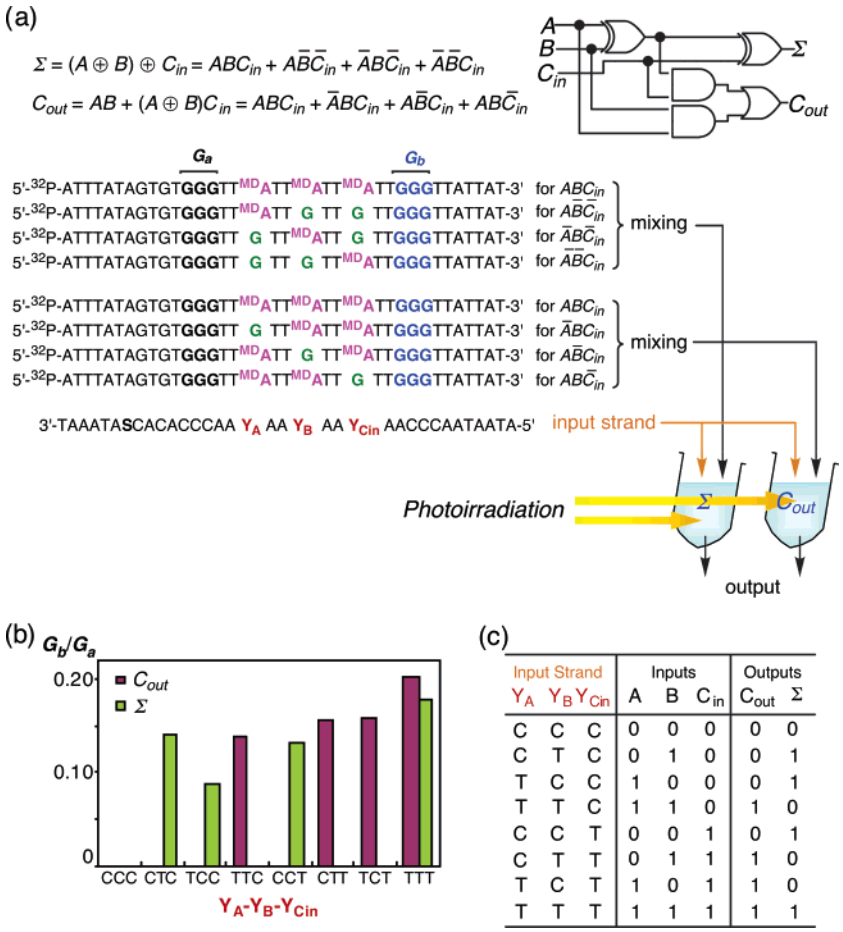
precipitated ODN was washed with 100 μL of 80% cold ethanol and dried in vacuo. The precipitated DNA was resolved in 50 μL of 10% piperidine (v/v), heated at 90 °C for 20 min, evaporated by vacuum rotary evaporation to dryness, and resuspended in 5–20 μL of 80% formamide loading buffer (a solution of 80% v/v formamide, 1 mM EDTA, 0.1% xylenecyanol, and 0.1% bromophenol blue). All reactions, along with Maxam–Gilbert G + A sequencing reactions, were conducted with heating at 90 °C for 1 min and quickly chilled on ice. The samples (1 μL, 3–10 kcpm) were loaded onto 15% denaturing 19:1 acrylamide–bisacrylamide gel containing 7 M urea and electrophoresed at 1900 V for approximately 1.5 h and transferred to a cassette and stored at −80 °C with Fuji X-ray film.

Results and Discussion

The synthesis of ^{MD}A-containing DNA was readily achieved from 4-chloro-6-methoxy-1*H*-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, 5′-d(CGCAAT^{MD}ATAACGC)-3′/5′-(GCGTTA**Y**ATTGCG)-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 DNA[14] and ionization potentials of hole-transporting bases[15] 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,[14a,17] were incorporated into both 5′- and 3′-sides of an ^{MD}A base. Hole transport was triggered by photoirradiation 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

(12) (a) Hunter, W. N.; Brown T.; Anand, N. K.; Kennard, O. *Nature* **1986**, *320*, 552–555. (b) Kalnik, M. W.; Kouchakdjian, M.; Li, B. F. L.; Swann, P. F.; Patel, D. J. *Biochemistry* **1988**, *27*, 100–108.
(13) Another 7-deazaadenine derivative, 2-amino-7-deazaadenine, is also known to form a stable wobble base pair with C. (a) Okamoto, A.; Tanaka, K.; Saito, I. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 97–99. (b) Okamoto, A.; Tanaka, K.; Saito, I. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3641–3643.
(14) (a) Saito, I.; Nakamura, T.; Nakatani, K.; Yoshioka, Y.; Yamaguchi, K.; Sugiyama, H. *J. Am. Chem. Soc.* **1998**, *120*, 12686–12687. (b) Nakatani, K.; Dohno, C.; Saito, I. *J. Am. Chem. Soc.* **2000**, *122*, 5893–5894.
(15) (a) Holmlin, R. E.; Dandliker, P. J.; Barton, J. K. *Angew. Chem. Int. Ed.* **1997**, *24*, 22715–2730. (b) Treadway, C. R.; Hill, M. G.; Barton, J. K. *Chem. Phys.* **2002**, *281*, 409–428.
(16) Nakatani, K.; Dohno, C.; Saito, I. *J. Org. Chem.* **1999**, *64*, 6901–6904.
(17) Okamoto, A.; Kanatani, K.; Taiji, T.; Saito, I. *J. Am. Chem. Soc.* **2003**, *125*, 1172–1173.



Input Strand Y_A	Y_B	Y_{Cin}	Inputs A	B	C_{in}	Outputs C_{out}	Σ
C	C	C	0	0	0	0	0
C	T	C	0	1	0	0	1
T	C	C	1	0	0	0	1
T	T	C	1	1	0	1	0
C	C	T	0	0	1	0	1
C	T	T	0	1	1	1	0
T	C	T	1	0	1	1	0
T	T	T	1	1	1	1	1

Figure 5. (a) Design of a DNA logic gate for a full-adder operation. Logic gate strands representing each product term were mixed with the Σ and C_{out} cuvettes. The samples in 10 mM sodium cacodylate (pH = 7.0) were photoirradiated (312 nm) at 0 °C for 45 min followed by a hot piperidine treatment (90 °C, 20 min). The results of the hole transport were analyzed as described in Figure 2. (b) Hole-transport efficiencies for each input strand. (c) Full-adder truth table. The strand cleavage patterns observed for each input strand were in good agreement with data shown in the truth table for the full adder.

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).[18] 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 (Y_A−Y_B). When the input strand where both input pyrimidines were T was used, the strand cleavage at the G_b site as an output signal was observed (G_b/G_a = 0.43), indicating that effective hole transport had occurred (Figure 4a). When the input pyridines Y_1 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.[19] 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

(18) (a) Boone, E.; Schuster, G. B. *Nucleic Acids Res.* **2002**, *30*, 830−837. (b) Giese, B. *Annu. Rev. Biochem.* **2002**, *71*, 51−70. (c) Bhattacharya, P. K.; Barton, J. K. *J. Am. Chem. Soc.* **2001**, *123*, 8649−8656. (d) Giese, B.; Wessely, S. *Angew. Chem., Int. Ed.* **2000**, *39*, 3490−3491. (e) Arkin, M. R.; Stemp, E. D. A.; Pulver, S. C.; Barton, J. K. *Chem. Biol.* **1997**, *4*, 389−400.

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;[19] 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 (^{MD}A and G as an inversion of ^{MD}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 (Σ and C_{out}) through AND, OR, and XOR operations.[19] 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 photoirradiation 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.

(19) *Digital Fundamentals*, 8th ed.; Floyd, T. L., Ed.; Pearson Education, Inc.: Upper Saddle River, NJ, 2003.

Conclusion

In conclusion, we have demonstrated the design and development of a new DNA logic gate. We indicated three important factors that are prerequisites for designing DNA logic gates: (i) logic bases, ^{MD}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 combinational 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.

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