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Molecular reactions for a molecular memory based on hairpin DNA

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Abstract

We have developed molecular reactions that can be used for a DNA molecular memory. The DNA molecular memory is a memory based on DNA molecules and their molecular reactions. The DNA molecule is used as a memory element that has address information in its base sequence and the molecular reaction is used for memory addressing and data writing. Because of using molecules and molecular reactions, the memory is able to provide a large amount of memory space and allows a massively parallel addressing without physical wiring. Here, we report on the DNA molecules and molecular reactions that can be used for constructing the molecular memory.

Key Words: molecular memory, DNA nanotechnology, DNA computer, molecular beacon, hairpin DNA

Area of Interest: Emerging New Technology

1. Introduction

A DNA molecular memory is a memory based on DNA molecules and their molecular reactions. The DNA molecule is a nano-scale memory element with address information in its base sequence. The molecular reaction, which is mainly a hybridization reaction or an enzyme reaction, is involved in memory operations. The molecular reactions of a large amount of DNA molecules proceed in parallel so that the DNA molecular memory is able to provide a large amount of memory space and allows a massively parallel addressing and writing without physical wiring.

To construct a memory, transitions between bistable states are generally required. The bistable states correspond to a written state and an unwritten state, respectively. We have realized the transitions between bistable states by molecular reactions based on hairpin DNA. Our DNA molecular memory is composed of two types of DNA: a hairpin DNA, which forms intramolecular base pairs, and a linear DNA, which does not form them. The hairpin DNA acts as a memory

molecule with a memory address, the linear DNA as a data molecule with an address tag of the memory. This memory exploits a hybridization reaction between the hairpin DNA and the linear DNA in memory addressing. Writing data on the memory is to make the linear DNA hybridize with the hairpin DNA. The hairpin DNA changes from a closed hairpin structure to an open structure when the data is written on the memory. Erasing data from the memory is to separate the linear DNA from the hairpin DNA. The hairpin DNA returns to the closed structure when the data is erased from the memory. The written and erased states are the bistable states at room temperature, and the transitions between the two states are realized through temperature control of the DNA memory solution.

In molecular biology, a hairpin DNA is used as a probe called a molecular beacon [1]. The molecular beacon exploits the structural change of hairpin DNA brought about by DNA hybridization as well as our molecular memory does. When the molecular beacon hybridizes with target DNA, the molecular beacon changes from a closed hairpin structure to an open structure and then reveals the presence of target DNA through fluorescence. The molecular beacon is similar to our molecular memory in terms of using structural change. However, the molecular beacon does not work as a memory because it does not involve transitions between bistable states.

Here, we report on the DNA molecules and molecular reactions that can be used for constructing the molecular memory.

2. Materials and Methods

2.1 Memory DNA and data DNA

The DNA molecular memory is composed of two types of DNA: a hairpin DNA and a linear DNA. We call the hairpin DNA the memory DNA and the linear DNA the data DNA based on their respective functions. A memory DNA with a sequence of 5'-TAMRA-GGACACGGTGCAGTGT AAGCAACTATTGTCTCCGTGTCC-Dabcyl-3' (Figure 1) was commercially synthesized and purified by HPLC (Qiagen, Tokyo, Japan). TAMRA is a fluorescent dye and Dabcyl is its quencher. Fluorescence emitted from TAMRA is quenched by Dabcyl only when TAMRA and Dabcyl are very close (within about a few nanometers). The Dabcyl-based quenching is known as non-FRET quenching, and it is often used in addition to FRET [2]. A data DNA with a sequence of 5'-GACACGGAGACAATAGTTGCTTACAC TGCA-3' (Figure 1) was commercially synthesized and purified by OPC (Qiagen). The data DNA has a complementary base sequence of the loop and the 3'-stem of memory DNA. A data-complementary DNA has a sequence of 5'-TGCAGTGTAAG CAACTATTGTCTCCGTGTC-3' (Figure 1), and was commercially synthesized and purified by OPC (Qiagen). The data-complementary DNA has a completely complementary base sequence of the data DNA.

2.2 Measurement of melting curves

A melting curve of memory DNA was obtained by monitoring TAMRA fluorescence at temperatures from 30 to 85 ºC. The temperature was increased at the rate of 1 ºC/min. The fluorescence of a 0.10 μM solution of memory DNA in 1×SSC (150 mM sodium chloride and 15 mM sodium citrate) was measured by using a fluorescence spectrometer LS55 (Perkin-Elmer, MA, USA).

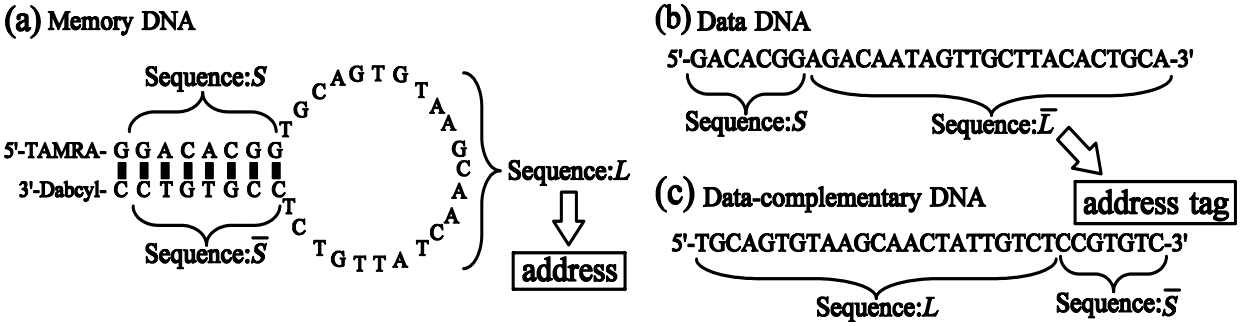

Figure 1. Memory DNA and Data DNA

(a) Memory DNA: a fluorescent dye TAMRA is attached to the 5'-end and its quencher Dabcyl is attached to the 3'-end. (b) Data DNA: a data DNA has a complementary base sequence of the loop and the 3'- stem of the memory DNA. (c) Data-complementary DNA: a data-complementary DNA has a completely complementary base sequence of the data DNA. $\bar{S}$ and $\bar{L}$ represent complementary base sequences of S and L, respectively.

The intrinsic fluorescence intensity of TAMRA varied with temperature. We therefore used the following relative fluorescence intensity F_R to exclude the temperature dependence:

$$F_R(T) = \frac{F_{obs}(T)}{F_{std}(T)}, \tag{1}$$

where T is temperature, and F_{obs} and F_{std} are the observed fluorescence intensity of TAMRA attached to memory DNA and free in solution, respectively. The concentration of TAMRA free in solution is the same as that of memory DNA. The following equation was used to normalize the fluorescence data:

$$F(T) = \frac{F_R(T) - F_{lower}(T)}{F_{upper}(T) - F_{lower}(T)}, \tag{2}$$

where F is the normalized fluorescence intensity and F_R is the relative fluorescence intensity. F_{lower} and F_{upper} are the lower and the upper baseline of F_R, which were determined by linear fitting of data at temperatures from 30 to 35 ºC and from 80 to 85 ºC, respectively.

A melting curve of the duplex of data DNA and data-complementary DNA was obtained by monitoring fluorescence of SYBR Green I (Molecular Probes, OR, USA) at temperatures from 55 to 78 ºC. The temperature was increased at the rate of 1 ºC/min. SYBR Green I intercalates specifically in double-stranded DNA and emits fluorescence. The fluorescence intensity of the duplex of 0.10 μM data DNA and 0.10 μM data-complementary DNA in 1×SSC with 0.5×SYBR Green I was measured by using a fluorescence detector of a real-time PCR system DNA Engine Opticon 2 (MJ Research, MA, USA). Equations (1) and (2) were used to analyze the fluorescence data, and the lower and upper baselines (F_{lower} and F_{upper}) were determined by linear fitting of data at temperatures from 55 to 60 ºC and from 76.5 to 78 ºC, respectively.

2.3 Measurement of time courses of hybridization

Time courses of hybridization between 0.10 μM memory DNA and 0.10 μM data DNA in 1×SSC were measured at temperatures 25, 45, 55, and 65 ºC by monitoring TAMRA fluorescence using the fluorescence spectrometer LS55 (Perkin-Elmer). The time when the solutions of the memory and the data DNA were instantaneously mixed was set at 0 s. Equation (1) was used to analyze the fluorescence data.

2.4 Writing and erasing experiments

In writing and erasing experiments, 0.10 μM of memory DNA and 0.10 μM of data DNA were mixed in 1×SSC at room temperature, and the initial fluorescence intensity of the solution was measured by the fluorescence spectrometer LS55 (Perkin-Elmer). The writing and erasing operations were repeated six times in total. The fluorescence intensity of the solution was measured just after the operations. Equation (1) was used to analyze the fluorescence data in all measurements.

3. Results

3.1 Structure and operation of DNA molecular memory

A DNA molecular memory consists of two types of DNA molecules: memory DNA and data DNA (Figure 1). The memory DNA is a hairpin DNA, the data DNA a linear DNA. The loop region of memory DNA has a memory address, which is recognized by the data DNA. The data DNA has an address tag part and an actual data content part. The address tag part is comprised of a complementary base sequence of the loop and the 3'-stem of memory DNA. The data content part can be comprised of any molecules, such as DNA/RNA, protein, metal nano-particles, and so on.

The writing operation makes the data DNA bind to the loop region of memory DNA through a series of operations: heating up a solution of memory DNA and data DNA from room temperature T_R (= 25 ºC) to the writing temperature T_W then cooling it down from T_W to T_R. At T_W, the data DNA hybridizes with the memory DNA because the memory DNA opens and the memory-data DNA duplex is stable (Figure 2).

The erasing operation makes the data DNA separate from the memory DNA through a series of operations: heating up the solution from T_R to the erasing temperature T_E and cooling it down quickly from T_E to T_R. The duplex of memory DNA and data DNA is completely dissociated at T_E. The quick cooling allows the memory DNA to close so that the data DNA can no longer access the memory DNA. Because the rate of intramolecular reactions is much faster than that of intermolecular reactions, the memory DNA close before the data DNA is bound. The erased state is a local minimum of a free energy surface so that a large amount of energy is necessary to reach the written state, which is a global minimum of the free energy surface. Once the memory DNA and the data DNA are separated, they do not hybridize until temperature is raised to T_W (Figure 3).

The written and erased states were observed by measuring the fluorescence of the memory DNA. The fluorescence is detected when data is written on the memory and is not detected when data is not written because the memory DNA has TAMRA and Dabcyl at the 5'- and 3'-ends, respectively, which are involved in the non-FRET detection.

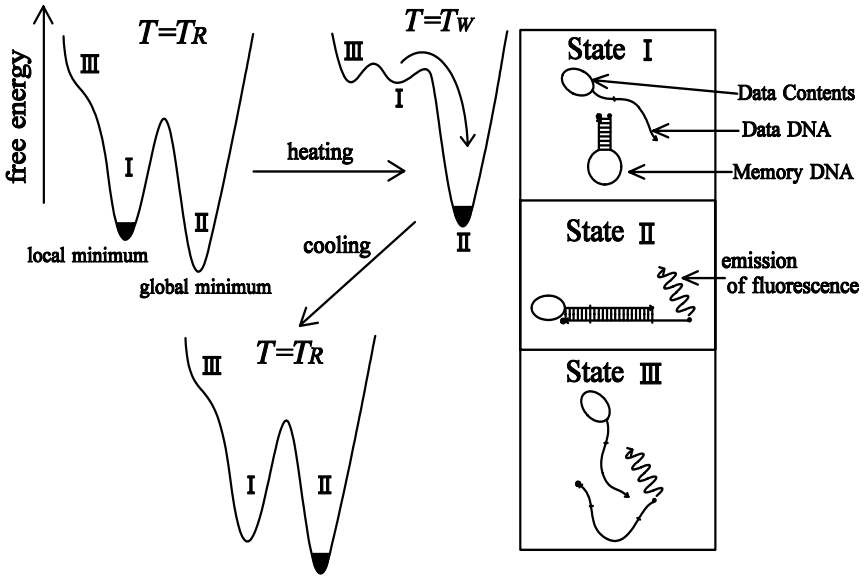

Figure 2. Writing

A series of operations composed of heating up a solution from T_R (room temperature) to T_W (writing temperature) then cooling it down from T_W to T_R. At T_W, the data DNA hybridizes with the memory DNA because the memory DNA opens and the memory-data DNA duplex is stable.

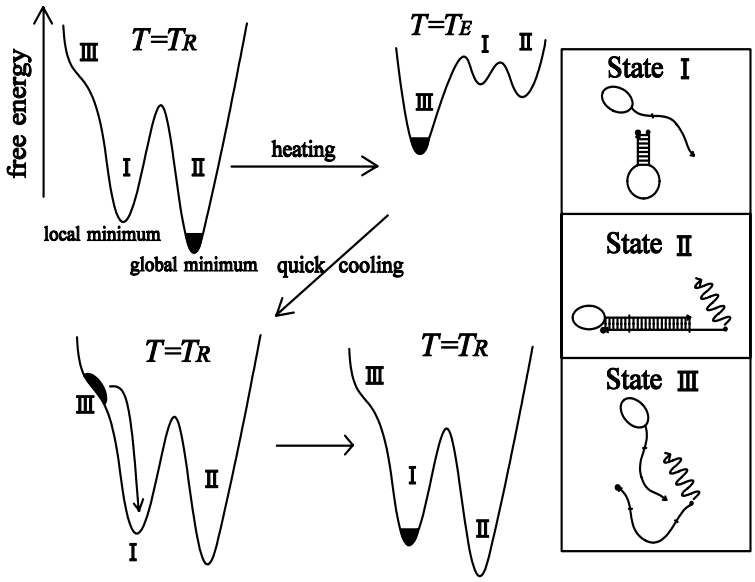

Figure 3. Erasing

A series of operations composed of heating up a solution from T_R (room temperature) to T_E (erasing temperature) and cooling it down quickly from T_E to T_R. The data DNA is completely dissociated from the memory DNA at T_E. The memory DNA closes after quick cooling so that the data DNA can no longer hybridize with the memory DNA.

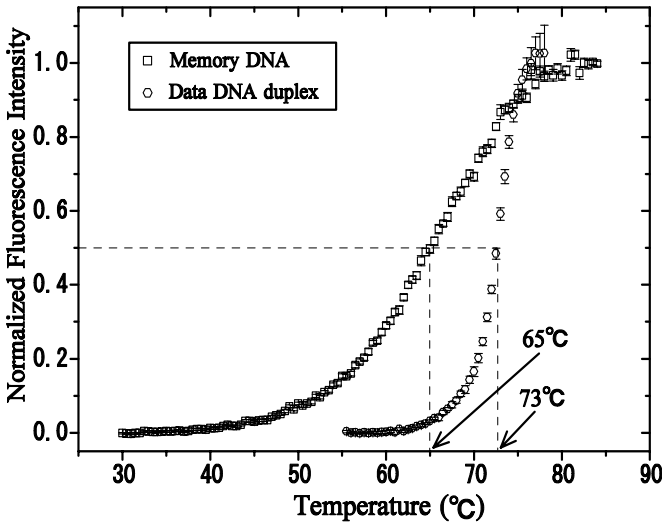

Figure 4. Normalized melting curves
The normalized melting curve of 0.10 μM memory DNA in 1×SSC (□) and that of 0.10 μM data DNA duplex in 1×SSC (○). Data represent the mean and standard error of three experiments.

3.2 Determination of writing and erasing temperature

The writing temperature T_W should be set at a temperature no lower than the melting temperature of the memory DNA and no higher than that of the memory-data DNA duplex because the memory DNA must be open and the memory-data DNA duplex must not be dissociated at the time of the writing. The erasing temperature T_E should be set at a higher temperature than the melting temperature of the memory-data DNA duplex because the memory-data DNA duplex must be completely dissociated at the time of the erasing. The melting temperature of the memory-data DNA duplex was estimated from the melting temperature of the data DNA duplex (the duplex of data DNA and data-complementary DNA) because the data-complementary DNA has an equivalent sequence of the hairpin DNA loop and the 3'-end side of the hairpin DNA stem.

Figure 4 represents the normalized melting curve of memory DNA and that of the data DNA duplex. The melting temperature of the memory DNA was 65 ºC. Based on the fact that the melting temperature of the data DNA duplex was 73 ºC, that of the memory-data DNA duplex was estimated to be 73 ºC. We therefore set T_W at 65 ºC and T_E at 75 ºC.

3.3 Determination of erasing rate

For the erasing operation to be successful, we must properly set the cooling rate, which is estimated from the kinetic properties of memory-data DNA hybridization. Figure 5 represents the time courses of memory-data DNA hybridization. The time to reach equilibrium was 7.0×10^4 s at 25 ºC, 1.5×10^4 s at 45 ºC, 2.0×10^3 s at 55 ºC, and 2.5×10^2 s at 65 ºC. The time to reach 50 % of equilibrium was about 20 s at 65 ºC. Therefore, the cooling rate V_E must be faster than 2.5 ºC/s: $(T_E - T_R)/(20\text{ s}) = (75\ ^\circ\text{C} - 25\ ^\circ\text{C})/(20\text{ s}) = 2.5\ ^\circ\text{C/s}$. Here, V_E was set at 2.5 ºC/s.

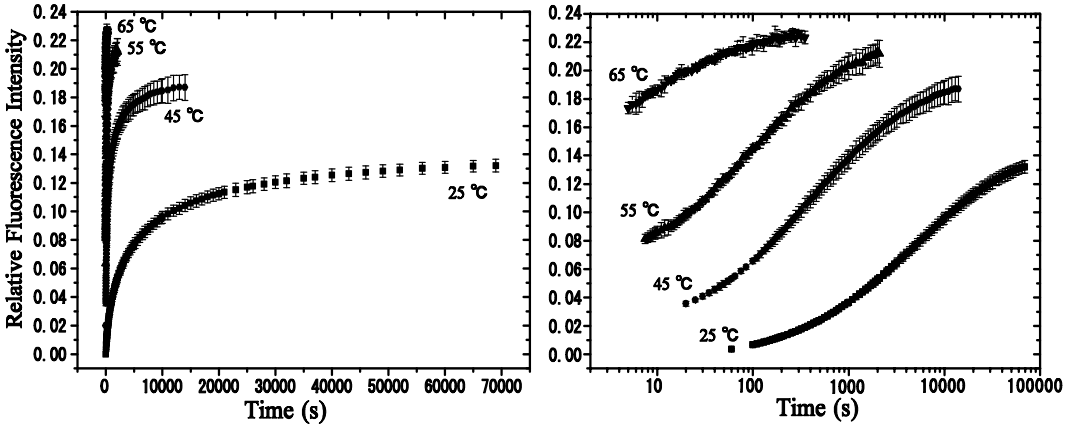

Figure 5. Time courses of hybridization

The time courses of hybridization between 0.10 μM memory DNA and 0.10 μM data DNA in 1×SSC at 25 (■), 45 (●), 55 (▲), and 65 ℃ (▼) are shown. The horizontal axis of the left-side panel is a linear scale and that of the right-side panel is a log scale. Data represent the mean and standard error of three experiments.

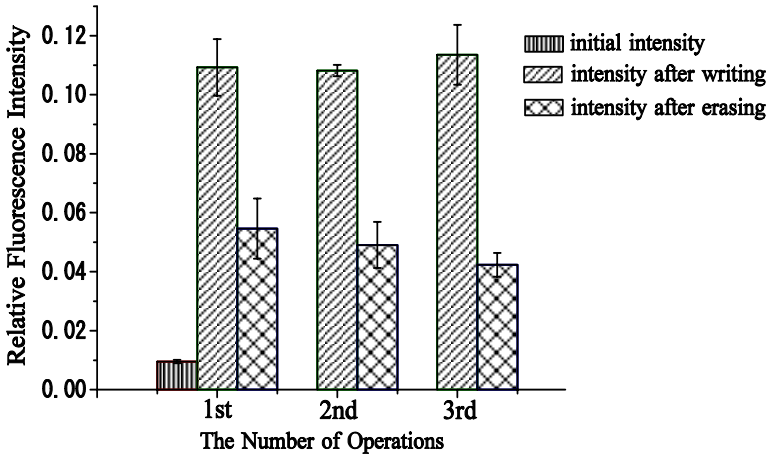

Figure 6. Writing and erasing of DNA molecular memory

The initial fluorescence intensity and the intensity after writing and erasing are shown in a bar chart. Data represent the mean and standard error of three experiments.

3.4 Writing and Erasing of DNA molecular memory

The experimental result of writing and erasing operations is shown in Figure 6. The fluorescence intensity increased after the writing operation, which means the writing operation made the data DNA bind to the memory DNA. The fluorescence intensity decreased after the erasing operation, which means the erasing operation made the data DNA separate from the memory DNA. Writing and erasing efficiencies did not change over three repetitions of these operations.

4. Discussion

The result of writing and erasing experiments demonstrates that the molecular reactions that can

be used for a DNA molecular memory work well repeatedly. The molecular reactions for addressing of a large amount of DNA molecular memories based on hybridization between the address part of hairpin DNA and the address tag part of linear DNA proceed in parallel so that massively parallel addressing of a huge memory space will be possible. The parallel addressing allows parallel writing, erasing and reading, which are greatly faster than sequential processing. To write a large amount of data in parallel by using the parallel addressing, a parallel generation of data contents is required. Combinatorial synthesis, for example, will allow the parallel generation of data DNA molecules. In addition, if the data DNA molecules can be distinguished one another by labels attached to them, we will be able to read out a large amount of data in parallel. Development of these techniques is the next step of the molecular memory research.

There are some problems that should be fixed to improve the performance of the DNA molecular memory. The first problem is that the data are not completely erased, which is due to the fact that the cooling rate of erasing is not fast enough to separate the memory DNA and the data DNA. We expect that faster cooling will allow data to be erased at higher efficiency. A local temperature control by laser beam, for example, will be able to increase the heating and cooling rates. The second problem is that the initial intensity is not zero, which is due to the fact that the data DNA hybridizes with the memory DNA even at 25 ºC. This problem can be circumvented by lowering the concentrations of memory and data DNA because their hybridization rate becomes slower at a lower concentration. To completely block their hybridization at room temperature, it is necessary to study the mechanism of memory-data hybridization in greater detail.

In this experiment, the memory DNA is free in a solution. This molecular memory will demonstrate higher abilities if fixed on a solid surface. The free DNA memory in solutions has only addresses at the loop of memory DNA but the fixed DNA memory will receive additional addresses based on positions on the surface. Application of the fixed DNA memory to an assembly technology will allow molecules to be manipulated on nanometer and micrometer scales. For example, if a functional molecule is used as the data content part and the memory DNA is attached to a solid surface, the data DNA with the functional molecule can be locally written on the memory DNA fixed on the surface by a local temperature control with a laser beam. The functional molecules can thus be arranged wherever we want to attach them on the surface by both light-directed addressing and molecular reaction addressing. This technology will provide a new strategy to manipulate molecules on both micro- and nano-scales.

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