An i-motif-containing DNA device that breaks certain forms of Watson–Crick interactions†

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An i-motif-containing DNA device is constructed that is able to break certain forms of Watson–Crick interactions under isothermal conditions.

Discovered in 1993,[1] the i-motif is a four-stranded assembly of two parallel duplexes of protonated cytosine–cytosine base pairs that are intercalated with each other in an antiparallel orientation.[2] Owing mainly to its specific self-recognition and susceptibility to pH variation, this cytosine-rich tetraplex has been utilized creatively as building components for fabricating various molecular devices and machines[3,4] in the past. The innovative inventions of these operable molecular architectures on the basis of i-motif[3,4] as well as other structural forms of DNA[5–7] inspired us recently to explore the possibility of using i-motif-embedded circular oligonucleotides in the design of new types of molecular nanostructures. We now report the synthesis and examination of a DNA device that could break apart certain forms of Watson–Crick interaction under isothermal conditions in its response to pH variation.

Fig. 1 shows a schematic representation of our approaches for designing a DNA device that breaks down the hydrogen bonds that uphold an 11-mer A- and T-rich duplex structure. Under neutral pH conditions, the two termini (Cytosine 1 and Cytosine 25) of a linear cytosine-rich oligonucleotide will position themselves randomly (***i*** in Fig. 1a) and will not be proximal to each other due to the torsional rigidity of DNA backbone.[8] Generation of a i-motif structure under acidic conditions,[9] however, will force Cytosine 1 and Cytosine 25 of this linear sequence to move toward each other in space (***ii*** in Fig. 1a). The first step of our approach in the current investigation is accordingly to covalently link the two termini of a 25-mer linear cytosine-rich sequence to an additional non-cytosine-rich segment (11-mer) to generate a backbone-circularized structural entity (36-mer, ***iii*** in Fig. 1b). At pH below 6.2, an i-motif structure is expected to form by the C-rich segment which would force Cytosine 1 and Cytosine 25 to move close to each other. This forced movement of Cytosine 1 and Cytosine 25 would consequently generate a backbone bending on the non-cytosine-rich segment (***iv*** in Fig. 1b). When the 11-mer non-cytosine-rich segment has formed a duplex structure with its complementary strand (Sequence 2) ahead of time (State 1 in Fig. 1c), the backbone bending on the A- and T-rich 11-mer associated with the formation of an i-motif structure would lead to the breakdown of hydrogen bonds between the two complementary 11-mer strands because there is absence of additional force to compel the backbone of Sequence 2 to bend along with the non-cytosine segment of Sequence 1. Since Sequence 2 is designed to hold the sequence of a molecular beacon (***v***), a stem-and-loop conformation (***vi***) will form as the single-stranded form is being generated.[7]

A circular oligonucleotide of 36-mer ($\langle C_1C_2C_3C_4T_5T_6T_7C_8C_9C_{10}C_{11}T_{12}T_{13}T_{14}C_{15}C_{16}C_{17}C_{18}T_{19}T_{20}T_{21}C_{22}C_{23}C_{24}C_{25}A_{26}A_{27}A_{28}A_{29}T_{30}T_{31}A_{32}A_{33}A_{34}A_{35}A_{36}\rangle$, Sequence 1) and a linear oligonucleotide of 19-mer tagged with BODIPY and DABCYL at its 5′ and 3′ ends (5′ BODIPY-ATATTTTTTAATTTTATAT-DABCYL 3′, Sequence 2) were accordingly designed during our investigation. Sequence 1 was synthesized in our laboratories through the ligation reaction of two termini of a linear 36-mer oligonucleotide (5′ TAAAAACCCCTTTCCCCTTTCCCCTTTCCCCAAAAT 3′, Sequence 3) catalyzed by T_4 DNA ligase

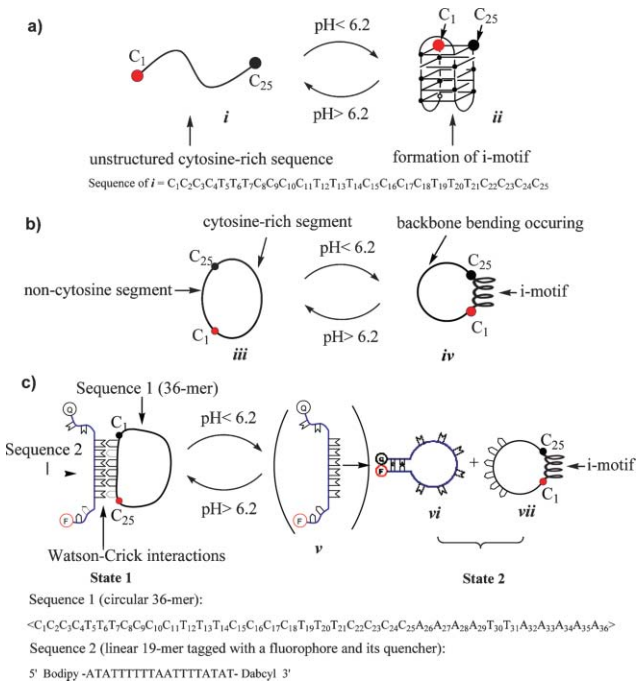

Fig. 1 Schematic representation of our approach for designing an i-motif-containing DNA device capable of breaking down certain forms of Watson–Crick interactions. a) Formation and disintegration of i-motif structure as the pH value of its environment varies. b) Formation and disintegration of i-motif structure that is embedded in a 36-mer circular oligonucleotide. c) Breakdown of the Watson–Crick interaction that upholds 11-mer T- and A-rich duplex segments. (see Supplementary Information† for detailed descriptions).

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(upper band in lane 2 in Fig. 2a). The circular nature of this newly formed oligonucleotide in its backbone was subsequently confirmed by examining its resistance to hydrolysis by exonuclease[10] (lane 4 in Fig. 2b). The maximum of fluorescence emission of BODIPY is known to occur at 515 nm when it is excited at 480 nm[11] and is insensitive to pH variation from 2 to 11.[12] A sample containing Sequence 1 and BODIPY-tagged Sequence 2 in an equal ratio was accordingly examined next at 20 °C using fluorescence spectroscopy. As shown in Fig. 3a, the emission intensity of this sample at 515 nm increased from 83 a.u. to 503 a.u. with the increase of pH from ~5.5 to ~7.5. These observations were consistent with the suggestion that Sequence 2 existed in a stem-and-loop conformation at pH ~ 5.5 (***vi*** in Fig. 1c) and was further transformed into a stem-open duplex structure with Sequence 1 at pH ~ 7.5 (State 1 in Fig. 1c). Subsequent variation of pH of the same system from ~7.5 to ~5.5 led to a decrease of emission intensity from 493 a.u. to 87 a.u. (Fig. 3b). This dramatic reduction of emission intensity suggests that the stem-open form of Sequence 2 was further transformed back to its stem-and-loop conformation (from State 1 to State 2 in Fig. 1c) under acidic conditions. This conformational alteration of Sequence 2 could be taken as the sign that a breakdown of the hydrogen bonds between Sequence 1 and Sequence 2 took place during the course of pH variation.

With the purpose of verifying that Sequence 1 was indeed separated from Sequence 2 at pH 5.5 (State 2 in Fig. 1c), a different test was carried out using a new 11-mer oligonucleotide

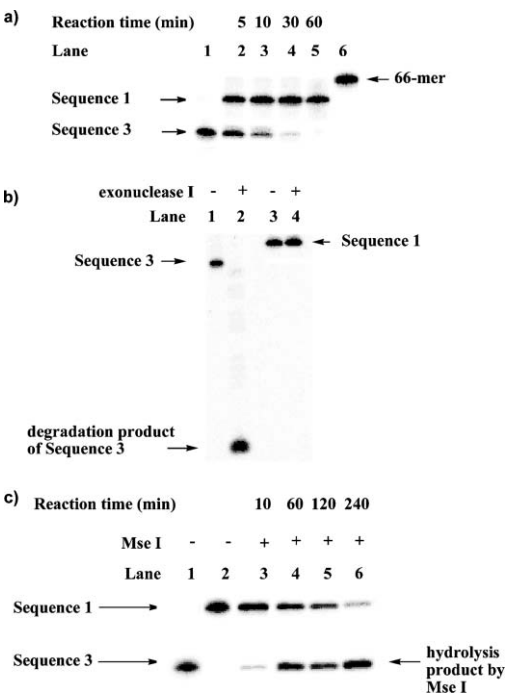

Fig. 2 Polyacrylamide gel electrophoretic analysis of oligonucleotides as components of the artificial DNA devices designed in the present studies. a) Preparation of Sequence 1 from its linear precursor on a 15-mer template catalyzed by T_4 DNA ligase. b) Confirmation of circularity of Sequence 1 in its backbone. c) Confirmation of presence of duplex structure between Sequence 1 and Sequence 2 (State 1 in Fig. 1c) at pH > 6.2. (see Supplementary Information† for detailed experimental descriptions).

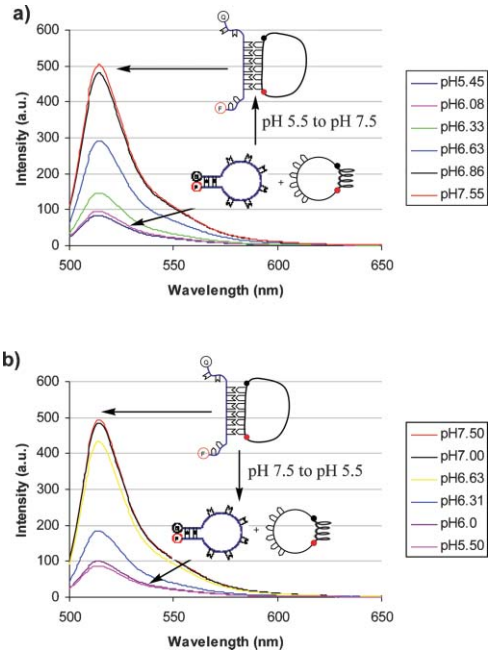

Fig. 3 Fluorescence spectroscopic analysis of formation and disintegration of duplex structure associated with the artificial devices designed in the current studies. a) Emission spectra of Sequence 1 and Sequence 2 as pH increases. b) Emission spectra of Sequence 1 and Sequence 2 as pH decreases (see Supplementary Information† for detailed experimental descriptions).

(5′ AAAATTAAAAA 3′, Sequence 5), which is complementary with Sequence 2. If Sequence 1 and Sequence 2 co-exist in a mixture in their single stranded forms (State 2 in Fig. 4a) as designed, addition of Sequence 5 to the mixture will in theory result in the formation of duplex structures between Sequence 5 and Sequence 2, thus leading to a dramatic increase of fluorescence emission. If Sequence 1 and Sequence 2 are present in a mixture in their duplex forms (State 1 in Fig. 4a), on the other hand, addition of Sequence 5 to the mixture will cause little change in the corresponding fluorescence emission. As shown in Fig. 4b, when Sequence 5 was added into a solution of Sequence 2 and Sequence 5 at pH 5.5, an instant and dramatic increase of fluorescence intensity of the sample was observed (Fig. 4b), which could be the indication that Sequence 2 pre-existed in a single stranded form in the mixture of Sequence 2 and Sequence 1 under an acidic condition.

Mse I is, on the other hand, a restriction endonuclease that recognizes TTAA-containing double helices of DNA and makes its incisions within this duplex segment.[13] To further prove that a duplex structure between Sequence 1 and Sequence 2 did actually exist under neutral condition (State 1 in Fig. 1c) as planned, a DNA cleavage test with Mse I was carried out during our investigations. As shown in Fig. 2c, the circular backbone of ^{32}P-labeled Sequence 1 was converted into its linear forms (lower bands in lanes 3 to 6) when Mse I was added to a mixture containing both Sequence 1 and Sequence 2 at pH 7. This observed strand scission is the indication of the presence of duplex structures in the designated system at pH 7 as expected (State 1 in Fig. 1c).

Since assembly and disassembly of i-motif and duplex structures of DNA were involved in the actions of the DNA device designed

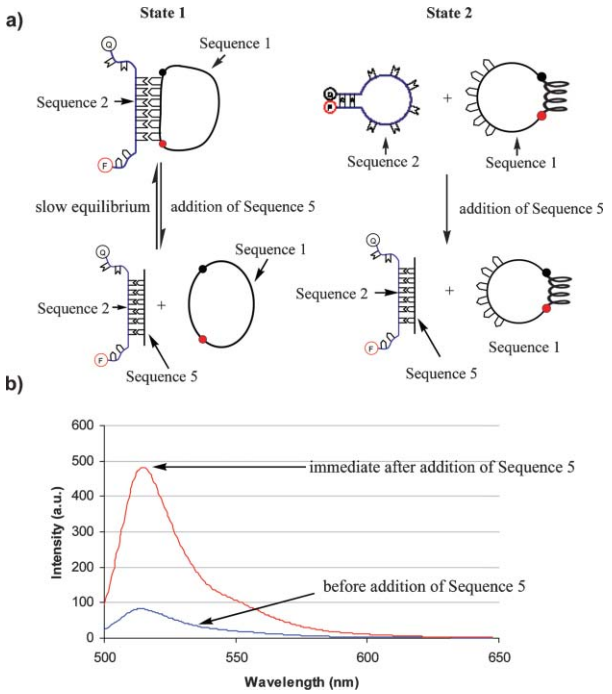

Fig. 4 a) Illustration of correlation among Sequence 1, Sequence 2 and Sequence 5 under different conditions. b) Emission spectra of Sequence 1, Sequence 2 and Sequence 5 in different combinations. A mixture containing 1 μM Sequence 1, 1 μM Sequence 2, 10 mM MES/HEPES (pH 5.5) and 1 M NaCl was prepared and its emission spectrum was recorded at 20 °C with excitation at 480 nm (blue). An equal amount of Sequence 5 to that of Sequence 1 was added next to the above solution and an emission spectrum of the new solution was further recorded under the same condition (red).

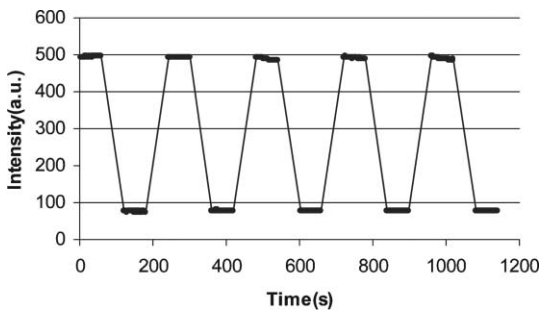

Fig. 5 Examination of operability of artificial DNA devices designed in the current studies using fluorescence spectroscopy. Changes of emission intensity with excitation at 480 nm were recorded as the designed DNA devices were operated for five cycles. (see Supplementary Information† for detailed experimental descriptions).

in the current studies, the time scales needed to complete each half of the cycle of the operation were measured next. The obtained response times of this DNA device to both pH decrease and increase were around 10 s (Fig. S1a and S1b), which matched those of similar operations reported in the literature.[3] Given that a relatively short time period was needed for each half of the cycle of the operation, this DNA device was further run in a periodic fashion. As shown in Fig. 5, decrease in fluorescence emission of the system is insignificant over five cycles, indicating that the accumulation of waste products generated from acid–base reactions has little effect on the hydrogen bonding breaking process in a limited number of cyclic operations, a phenomenon that has been observed in similar systems reported previously.[3]

In conclusion, when formation and disintegration of i-motif take place in its response to pH alteration, the relative displacement of two termini of the corresponding cytosine-rich oligonucleotide alters. A circular oligonucleotide containing such an i-motif structure was synthesized and its application in the design of a molecular device was examined in our studies. It is our expectation that the outcome of the current studies could serve as useful information in the future design of some new nanomolecular architectures.

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