# Single-Molecule Manipulation of the Duplex Formation and Dissociation at the G-Quadruplex/i-Motif Site in the DNA Nanostructure

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ABSTRACT We demonstrate the single-molecule operation and observation of the formation and resolution of double-stranded DNA (dsDNA) containing a G-quadruplex (GQ) forming and counterpart i-motif forming sequence in the DNA nanostructure. Sequential manipulation of DNA strands in the DNA frame was performed to

prepare a topologically controlled GQ/i-motif dsDNA. Using strand displacement and the addition and removal of K^+, the topologically controlled GQ/i-motif dsDNA in the DNA frame was obtained in high yield. The dsDNA was resolved into the single-stranded DNA, GQ, and i-motif by the addition of K^+ and operation in acidic conditions. The dissociation of the dsDNA under the GQ and i-motif formation condition was monitored by high-speed atomic force microscopy. The results indicate that the dsDNA containing the GQ- and i-motif sequence is effectively dissolved when the duplex is helically loosened in the DNA nanoscaffold.
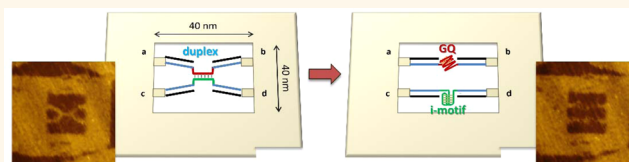
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Tandem G-rich repeat sequences, which form G-quadruplex (GQ) structures, are often observed in the promoter regions.[1] The GQ is thought to be closely involved in biological functions such as the regulation of gene expression and cell fate control.[2] Promoter sequences such as the *c-myc* promoter contain the G-rich sequence, which forms a GQ structure that is involved in the regulation of transcription.[2] In the double-stranded DNA (dsDNA) in this region, the complementary sequence contains a C-rich repeat sequence, which forms an i-motif structure.[3] A number of studies have reported on the formation of the GQ and/or i-motif in the promoter regions containing GQ- and i-motif-forming sequences.[4−7]

The i-motif structure is physically induced in an acidic condition because the i-motif formation requires a hemiprotonated cytosine dimer formation.[3] However, when using some C-rich sequences, such as the proximal promoter region of the RET onco-gene, the i-motif structure can be formed close to a neutral pH condition.[8] In addition, i-motif formation at neutral pH has also been observed in a dsDNA with negative superhelicity[5] and in a molecular crowding environment.[9] A promoter sequence such as the insulin-linked polymorphic region (ILPR) containing 5′-ACA(GGGGTGT)$_4$-3′ has been investigated, and the unfolding and folding of the GQ and i-motif in the ILPR sequence has been investigated at the single-molecule level using optical tweezers by measuring the unfolding force by mechanical unfolding of the structures under different pH and ionic conditions.[6,7,10]

These unique structures have been recently used as sensors for the detection of DNA molecules and molecular switching devices that are responsive to ions and pH.[11−13] The manipulation of these structures is also

an interesting target for the construction of structure-based sensors in nanotechnology.[11−13]

Using DNA nanostructures and high-speed atomic force microscopy (AFM) imaging techniques, direct observation of the DNA structural changes at the single-molecule level has been recently reported.[14] In these experiments, the individual target DNA structures to be observed were assembled in a frame-like DNA nanostructure, called a DNA frame, and the movement of the DNA strands containing unique sequences in the DNA frame was investigated. For example, single-molecule G-multiplex structures and their reconfiguration were visualized in the DNA frame,[15−18] and the B−Z transitions,[19,20] and the imaging of association and dissociation of photoresponsive DNA strands[21−23] were demonstrated. The enzyme behaviors and reactions in the DNA frame structures were also investigated using the designed nanostructures and high-speed AFM.[24−27]

In this study, we manipulated the formation and dissociation of the GQ/i-motif complementary sequence in the ILPR core sequence in the DNA frame structure (Figure 1). The ILPR promoter region is considered to be separated into the GQ and i-motif structures in the regulation of transcription.[7] The GQ/i-motif sequence was introduced into the DNA frame for observation of the GQ and i-motif formation under the various conditions. In this system, topologically controlled dsDNA was prepared using the sequential manipulation of the interaction of DNA strands in a series of programmed operations. We performed the resolution of dsDNA containing the GQ/i-motif sequence by controlling the pH and K^+ conditions, which were changed individually or together during the formation of the GQ and i-motif. Furthermore, these structural changes were directly observed by high-speed AFM at the single-molecule level.

RESULTS AND DISCUSSION

GQ and i-Motif Formation in the DNA Frame. We examined the GQ and i-motif formation in a pH 7.6 or 5.5 solution containing 50 mM KCl. CD spectral measurements were employed to investigate the formation of the GQ and i-motif structures using individual GQ- and i-motif-forming sequences.[28] In the case of the GQ formation, the CD band specific for the GQ increased in the presence of 50 mM KCl, indicating that the GQ structures formed at both pH 7.6 and 5.5 (Figure 2a). The CD bands show the characteristics of a mixture of parallel ($\lambda_{max} = 265$ nm; $\lambda_{min} = 238$ nm) and antiparallel ($\lambda_{max} = 293$ nm) GQ structures (Figure 1c).[28] In the case of the i-motif formation, the CD band specific for the i-motif ($\lambda_{max} = 290$ nm; $\lambda_{min} = 263$ nm) appeared at pH 5.5 in both the absence and presence of KCl, indicating that the i-motif formed in the acidic condition (Figure 2d).[6,8] Next, we examined the GQ and i-motif formation in the DNA nanostructure. We divided the GQ-forming sequence into two fragments and introduced them in the middle of the two supporting dsDNAs in the DNA frame (Figure 2b and Figure S1).[15a] When the GQ formed in the center, two separated supporting dsDNAs should be connected and would be observed as an X-shape in the DNA frame (Figure 2b). Before the addition of KCl, two dsDNAs were clearly separated in the AFM images. After the addition of KCl, the X-shaped dsDNAs appeared, indicating the formation of the GQ structure in the DNA frame. The yield of the X-shape formation in the presence of K^+ was 68%, while in the initial state, the separated structure formed in 93% yield (Figure 2c).
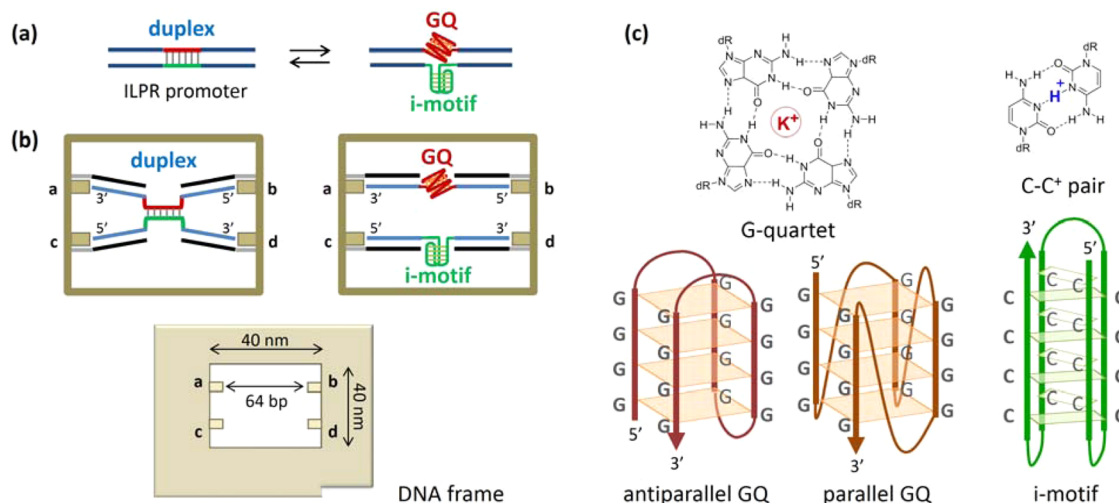

Figure 1. Single-molecule observation of the GQ and i-motif formation from a duplex in the DNA frame. (a) The ILPR promoter region is thought to be separated into the GQ and i-motif in the regulation of transcription. (b) The GQ- and i-motif-forming core sequences of the ILPR promoter are introduced into the DNA frame shown below for observation of the GQ and i-motif under various conditions. (c) G-quadruplex (antiparallel and parallel GQ) and i-motif structure. For the formation of the GQ and i-motif, K^+ and acidic conditions are required, respectively.
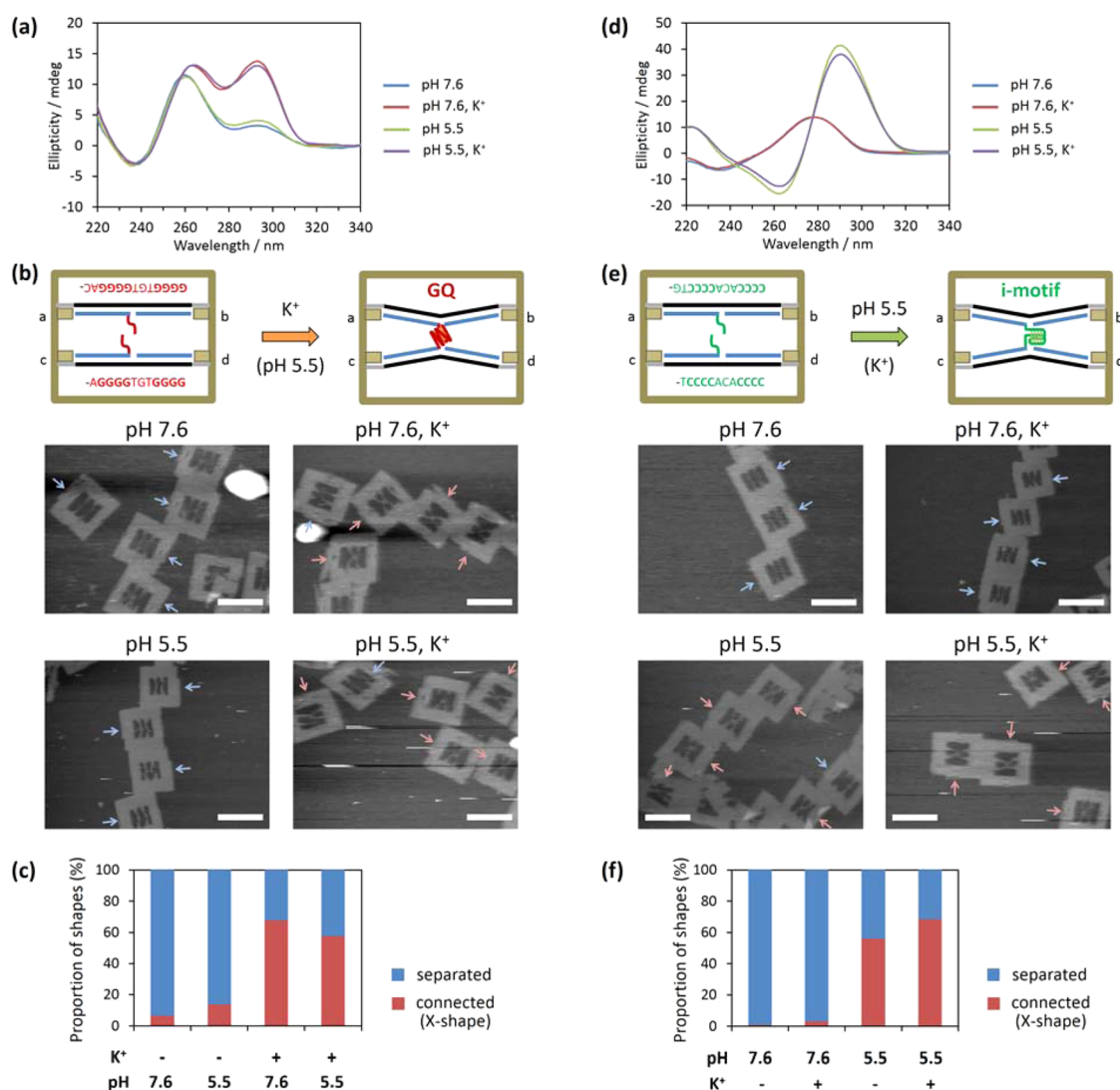
Figure 2. Single-molecule GQ and i-motif formation in the observation conditions. (a) CD spectra of GQ in the various conditions. (b) AFM images of DNA frames containing G-tracts for the interstrand GQ formation in the presence of K^+. Blue and red arrows indicate the separated and connected (X-shaped) strands, respectively. Scale bars: 100 nm. (c) Summary of the interstrand GQ formation in the DNA frame. (d) CD spectra of the i-motif in the various conditions. (e) AFM images of DNA frames containing C-tracts for the interstrand i-motif formation in the acidic condition. Blue and red arrows indicate the separated and connected (X-shaped) strands, respectively. (f) Summary of the interstrand i-motif formation in the DNA frame.

In addition, using the acidic condition at pH 5.5 with K^+, GQ formation was still observed in 58% yield. The formation of the GQ was clearly induced by the addition of K^+ in the DNA frame.

We also examined the i-motif formation in the DNA frame. Using the divided i-motif-forming sequence, these two short strands were introduced between the two supporting dsDNAs (Figure 2e and Figure S1). When the i-motif is formed in the center, two separated strands should be connected and observed as an X-shape in the DNA frame (Figure 2e). At pH 7.6, two dsDNAs were clearly separated and had a yield of 99% in the AFM images. After changing to the pH 5.5 condition, X-shaped dsDNAs appeared in 56% and 69% yield in the presence and absence of K^+, respectively. These results indicate that the formation of the

GQ and i-motif can be controlled in the DNA frame by altering the K^+ and acidic condition. We further used the conditions obtained in the bimolecular GQ and i-motif formation for the intrastrand GQ and i-motif formation from the GQ/i-motif duplex in the DNA frame.

Operation to Prepare a Topologically Controlled dsDNA in the DNA Frame. We next constructed a topologically controlled dsDNA in the DNA frame. To observe the resolution of the dsDNA containing a GQ/i-motif sequence in the DNA frame, two strands should be introduced into the DNA frame separately. If the two strands are incorporated into the DNA frame at the same time, the complementary GQ/i-motif parts hybridize to form a double helix. Therefore, if the hybridized GQ/i-motif duplex is introduced into the DNA

frame, the locked state of the duplex that is not resolved into GQ and i-motif strands should be preserved (described later). As shown in Figure 3a, we introduced GQ-forming single-stranded DNA (ssDNA) and i-motif-forming ssDNA separately into the top and bottom sides of the DNA frame, respectively (Figure S3). Here we used the core sequence of the GQ- and i-motif-forming sequence in the ILPR promoter. Using these sequences, we confirmed the usual GQ and i-motif formation in the CD spectra as reported previously (Figure 2a and d).[6,28] We introduced them sequentially using a stepwise operation. First, the DNA strand with the i-motif sequence was introduced into the bottom side of the DNA frame (state A). Here, the i-motif sequence was covered with a toehold-containing complementary strand to prevent the binding to the GQ ssDNA in the next step. The i-motif sequence covered with a complementary strand having a toehold part was incorporated into the bottom side. Next, a GQ-forming DNA strand was incorporated into the top side in the DNA frame (state B). Here, the strand containing the GQ structure in the presence of K^+ was incorporated into the DNA frame to avoid strand exchange between the protection strand of the i-motif sequence and GQ-forming strand. In the AFM image,

the two separated strands were observed in the DNA frame in 81% yield (Figure 3b left and Figure S4a).

In the third step, the toehold-containing protection strand was removed from the bottom strand by strand displacement using a fully complementary strand (state C). After the strand displacement in the presence of K^+, two strands remained unbound in 76% yield, as shown in the previous step (Figure 3b center and Figure S4b). This indicates that the GQ structure prevented binding to the complementary i-motif-forming sequence.

Finally, K^+ was removed by ultrafiltration to induce the formation of GQ/i-motif dsDNA (state D). In the AFM image, most of the supporting dsDNAs in the DNA frame formed the X-shape (Figure 3b right and Figure S4c), indicating that the GQ- and i-motif ssDNA hybridized to form a duplex. The yield of GQ/i-motif duplex formation was 89% (Figure 3c). Using these operations, we successfully prepared the topologically controlled GQ/i-motif dsDNA in the DNA frame. The prepared GQ/i-motif duplex could be used for the resolution of the duplex into the GQ and/or i-motif structure under the GQ- and i-motif-forming conditions.

Hybridization of the GQ ssDNA and i-motif ssDNA into the GQ/i-motif duplex was observed by high-speed
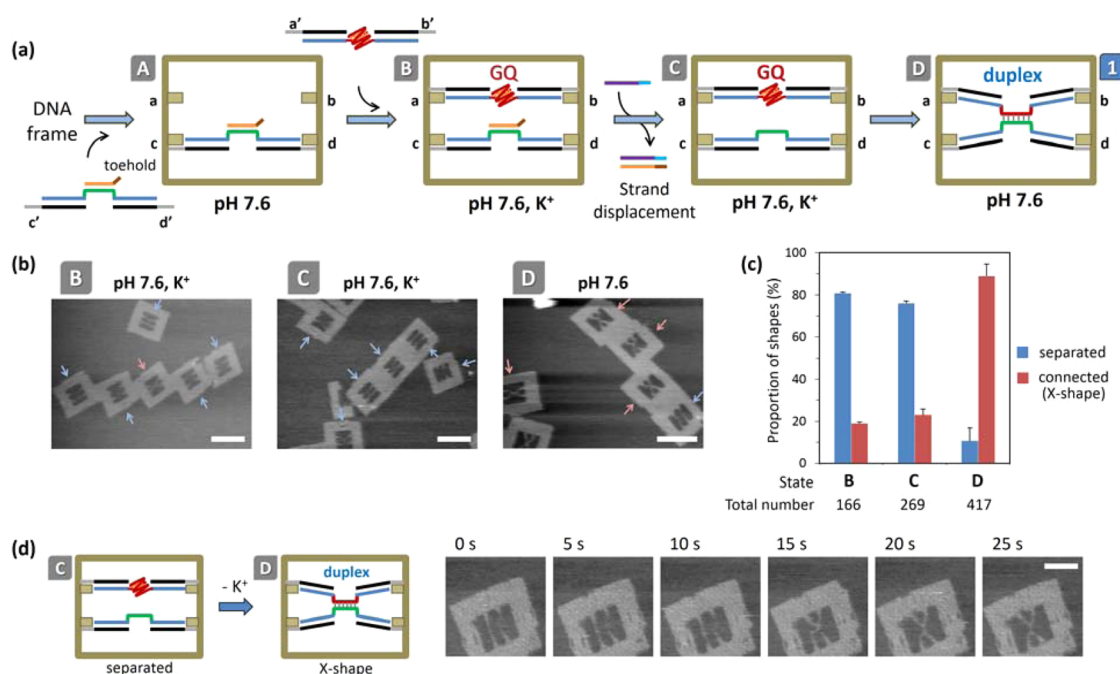

Figure 3. Operation to prepare topologically controlled GQ/i-motif dsDNA in the DNA frame. First, a DNA strand that had its i-motif sequence [5′-CCCCACACCCCTGTCCCCACACCCC-3′] covered with a toehold-containing complementary strand was incorporated into the bottom side (c and d connector) in the DNA frame (state A). Second, a DNA strand that formed the GQ [5′-GGGGTGTGGGGACAGGGGTGTGGGG-3′] was incorporated into the top side (a and b connector) in the DNA frame (state B). Third, the complementary toehold strand was introduced to uncover the i-motif sequence (state C). Fourth, K^+ was removed to hybridize two strands to form dsDNA (state D). (b) AFM images of the structures, states B, C, and D. Blue and red arrows indicate the separated and connected (X-shaped) strands, respectively. Scale bars: 100 nm. (c) Summarized results of the formation of separated and connected DNA strands in the DNA frame. Blue and red bars indicate the separated and connected (X-shaped) strands, respectively. The values (mean ± SD) were obtained from three independent experiments. (d) Single-molecule observation of GQ and i-motif formation from the duplex in the DNA frame. Time-lapsed AFM images of the formation of the dsDNA induced by removal of K^+. Time 0 s is an arbitrary time during the AFM scanning. Scanning rate, 0.2 frames/s. Scale bar: 50 nm.

AFM (Figure 3d). Two separated strands in the DNA frame (state C to D) in the presence of K^+ were adsorbed onto mica, and AFM imaging was performed in the buffer without K^+. During AFM scanning, two separated strands were connected to form an X-shaped structure. At 15 s, two strands were partially attached, and at 20 s, these were connected and hybridized, which was observed as a single line in the middle. The hybridized strand never reverted to two strands. These results suggest that the process for the X-shape formation was initiated by disruption of the GQ structure by removal of K^+ and the subsequent hybridization of the GQ- and i-motif-forming ssDNAs.

Manipulation of the Topologically Controlled dsDNA to Resolve into the GQ and i-Motif in the DNA Frame. To investigate the resolution of the ILPR promoter domain containing the GQ- and i-motif-forming sequence, the prepared GQ/i-motif duplex was dissociated into two strands under the GQ- and i-motif-forming conditions (Figure 4a). We used the three conditions described in the first section (Figure 2): (i) GQ-forming condition (pH 7.6, K^+; state 1 to 2); (ii) i-motif-forming condition (pH 5.5; DNA state 1 to 3); and (iii) GQ- and i-motif-forming condition (pH 5.5, K^+; state 1 to 4) (Figure 4a). In the first operation (i), the duplex in the initial state (state 1), which formed in 91% yield, was treated with K^+-containing buffer at pH 7.6. In this condition, the GQ formation should resolve the GQ/i-motif duplex. As observed in AFM images, 51% of the structures separated into two strands (Figure 4b and Figure S5a). This result indicates that the formation of the GQ structure can induce the resolution of the manipulated GQ/i-motif duplex under the GQ-forming condition.

In the second operation (ii), the pH 5.5 condition was used to induce the formation of the i-motif structure. In this condition, the separated dsDNAs had a 38% yield (Figure 4b and Figure S5b). This result indicates that the formation of the i-motif structure can also solve the GQ/i-motif duplex.

In the third operation (iii), the GQ/i-motif duplex was exposed to the acidic condition in the presence of K^+. The duplex also separated into two dsDNAs in the DNA frame (Figure 4b and Figure S5c). The yield of the separated strands increased to 58%. In this condition, the GQ and i-motif formation occurred at the same time, and the separated strands were observed clearly, because the formation of the GQ and i-motif structure should tighten the individual strands. These results indicate that the GQ and i-motif formation induces the resolution of the GQ/i-motif dsDNA formed in the DNA frame. The yield of resolution of the GQ/i-motif duplex is still moderate (50−60%) at rt, so that, to promote the dissociation of the duplex and formation of the GQ and i-motif structure, the other factors such as temperature need to be examined further.

From these results, incubation with K^+, which induces the formation of the GQ structure, always promotes the resolution of the GQ/i-motif duplex. On the other hand, the i-motif formation needs the acidic condition, which is unlikely to occur in the promoter region in the transcription. For the i-motif formation, the pH should be kept low because protonation to cytosine is required. In the CD spectra of the i-motif-forming strand, the i-motif formation and deformation occurred at slightly acidic conditions ranging between pH 6.0 and 6.5 (Figure S6a). We also examine the pH-induced separation of the GQ/i-motif duplex at pH 6.0 and 6.5. In the pH 6.0 buffer, the GQ/i-motif duplex was separated into two strands, indicating that the formation of the i-motif induced the duplex dissociation (Figure S6b). On the other hand using pH 6.5 conditions, the GQ/i-motif duplex was not resolved (Figure S6c). These results suggest that i-motif formation can resolve the GQ/i-motif duplex at slightly acidic conditions, which should depend on the GQ/i-motif sequence in the promoter domain and environment such as crowding conditions.[9]

The GQ/i-motif duplex prepared in the DNA frame is a topologically controlled duplex. Two topologically separated ssDNA rings containing the GQ and i-motif sequence hybridized to form the GQ/i-motif duplex in the DNA frame. Here, single-stranded linkers connecting GQ- (or i-motif-) forming strands and supporting dsDNAs were added to hybridize the GQ-/i-motif-forming strands (Figures 3a and S3). When the GQ/i-motif duplex is formed, the GQ/i-motif duplex should be pulled by the single-stranded linkers from four terminals, which should work as a torque for unwinding the formed double helix. Therefore, this GQ/i-motif duplex should contain negative helicity to loosen the duplex. As reported previously, the GQ/i-motif double-helical domain with negative helicity promotes the dissociation of the duplex in the formation of the GQ structure.[5] In our system, resolution of the duplex can be easily induced in the K^+ and acidic condition. Therefore, using this topologically controlled duplex, the torque to loosen the double helix effectively worked to resolve the GQ/i-motif duplex coupled with the formation of the GQ and i-motif structure.

The advantage of using this topologically controlled GQ/i-motif duplex was proved by comparing with the usually formed duplex placed in the DNA frame. The GQ/i-motif duplex was introduced into the DNA frame just by annealing without using a stepwise operation as shown in Figure 3. The usually formed GQ/i-motif duplex was not resolved into two separated strands in the K^+ and acidic condition (Figure S7). As previously described once the usual duplex is fixed in the DNA frame, the duplex cannot resolve into the GQ and i-motif ssDNA. These results indicate that the topological control in the DNA frame suitably works to prepare a model of the GQ/i-motif duplex occurring in the promoter region.
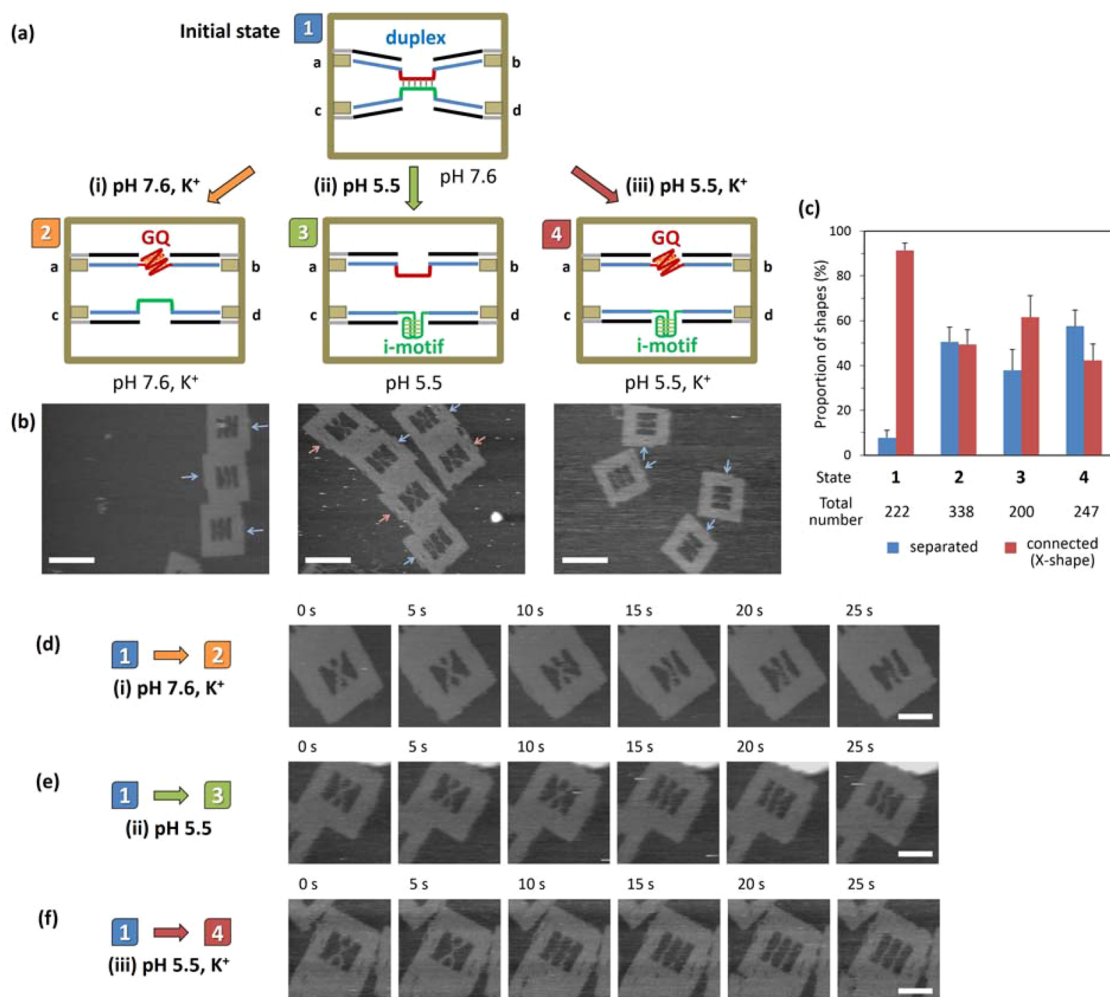
Figure 4. Resolution of the prepared GQ/i-motif dsDNA in the DNA frame into the G-quadruplex and i-motif structure.
(a) Three operations were used to change the states of DNA strands: (i) pH 7.6, K$^+$ for the GQ formation (state 1 to 2); (ii) pH 5.5 for the i-motif formation (state 1 to 3); and (iii) pH 5.5, K$^+$ for the GQ and i-motif formation (state 1 to 4). (b) AFM images for the three conditions for formation of states 2−4. Blue and red arrows indicate the separated and connected (X-shape) strands, respectively. Scale bars: 100 nm. (c) Summarized results of formation of separated and contacted DNA strands in the DNA frame using these operations. The values (mean ± SD) were obtained from three independent experiments. Time-lapsed high-speed AFM images for the separation of the GQ/i-motif dsDNA induced by the K$^+$ and acidic condition in the DNA frame. (d) Time-lapsed images of the dissociation of duplex in the presence of K$^+$. (e) Time-lapsed images of the dissociation of duplex under the acidic condition. (f) Time-lapsed images of the dissociation of duplex in the presence of K$^+$ under the acidic condition. Time 0 s is an arbitrary time during the AFM scanning. Scanning rate, 0.2 frame/s. Scale bars: 50 nm.

High-Speed AFM Observation of the Resolution of the GQ/i-Motif Duplex in the DNA Frame. We used high-speed AFM to further examine the separation of the GQ/i-motif duplex at the single-molecule level (Figure 4d−f). We prepared samples containing the GQ/i-motif duplex and kept them in pH 7.6 buffer in the initial condition. The duplex samples were then observed by high-speed AFM under the three different conditions shown in Figure 4a. The prepared samples were adsorbed onto a mica plate and then observed in the observation buffer containing K$^+$ and pH 5.5 buffer.

First, we examined the separation in the GQ-forming condition with K$^+$ and pH 7.6 buffer. The X-shaped structure in the parallel conformation changed into two separated strands during AFM scanning (Figure 4d). The separation of the dsDNA and the corresponding conversion of the X-shaped structure into the separated strands occurred at 15 s. The separated dsDNA never reverted to form the X-shape. This observation suggests that the resolution of the GQ/i-motif duplex was induced by the GQ formation followed by separation of the strands.

Next, using the acidic condition, we examined the separation of the dsDNA in the DNA frame. The structural conversion from the X-shaped structure to the two separated strands was observed at 15 s (Figure 4e). The separated dsDNA also never reverted to form the X-shape, which suggests that the resolution of the GQ/i-motif duplex can also be induced by the i-motif formation, and the subsequent separation of the strands occurred.

Finally, we examined the dynamic movement of DNA strands in the acidic condition with K^+. We observed the clear structural conversion from the X-shaped structure to the two separated strands at 10 s (Figure 4f). The separated dsDNA appeared as straight strands because both strands should form the GQ and i-motif structures. This also suggests that both the GQ and i-motif formation strongly induced the resolution of the GQ/i-motif duplex. These results indicate that the dynamic dissociation of the GQ/i-motif duplex induced by the GQ and i-motif formation can be observed at single-molecule resolution using this topologically controlled system and high-speed AFM. Although it is still difficult to obtain the time constant for the resolution of the duplex, the resolution occurred within 5 s during one-time AFM scanning for this high-speed AFM observation. The DNA frame should easily loosen the GQ/i-motif duplex compared with the usual dsDNA and alter the equilibrium between the dsDNA and ssDNAs, so that the DNA frame can promote the dissociation of the dsDNA into ssDNAs, and the formed ssDNAs subsequently changed into the stable GQ and i-motif depending on the buffer conditions.

The reverse hybridization to form the GQ/i-motif dsDNA (state 1 in Figure 4 and state D in Figure 3) from the separated GQ structure and i-motif strand (state 2 in Figure 4 and state C in Figure 3) was imaged by high-speed AFM by removing K^+ (Figure 3d). The reversible formation and dissociation of the GQ/i-motif dsDNA can be manipulated by the stepwise operations.

CONCLUSIONS

We have demonstrated the formation and dissociation of dsDNA containing the GQ sequence and the counterpart i-motif sequence in the DNA nanostructure and observed them at the single-molecule level. We performed sequential manipulation of DNA strands in the DNA frame and successfully prepared the topologically controlled GQ/i-motif dsDNA. Using the toehold strand and the addition and removal of K^+, the topologically controlled GQ/i-motif dsDNA in the DNA frame was obtained in high yield. Using this prepared GQ/i-motif duplex, the dsDNA was resolved into the GQ and the i-motif by addition of K^+ and/or under an acidic condition as indicated in the promoter region. The dissociation of dsDNA after the formation of the GQ and i-motif was monitored by high-speed AFM, and the dissociation of dsDNA containing the GQ- and i-motif-forming sequence was controlled in the DNA frame. The results obtained in this study show that the GQ/i-motif duplex system constructed in the DNA frame can be a structural model for studying the dynamic GQ and i-motif formation, which is involved in the regulation of gene expression in the promoter region.

EXPERIMENTAL SECTION

Materials. Single-stranded M13mp18 viral DNA was purchased from New England Biolabs, Inc. (Ipswich, MA, USA). All the staple DNAs for the DNA frame were purchased from Eurofins Genomics (Tokyo, Japan). The DNA strands for incorporation into the DNA frame were purchased from Japan Bio Services (Saitama, Japan). The gel-filtration column was prepared with Sephacryl-400 (GE Healthcare) with the experimental buffer. Superfiltration was performed using a superfiltration column (Amicon). Circular dichroism (CD) spectra were obtained using a JASCO J-1100 spectropolarimeter (Tokyo, Japan) in a solution containing DNA strands (5 μM), 10 mM Tris (pH 7.6) or MES (pH 5.5) buffer, 12.5 mM MgCl$_2$, and 1 mM EDTA in the presence or absence of 50 mM KCl.

Single-Molecule GQ and i-Motif Formation in the DNA Frame. The DNA origami frame was prepared by annealing the solution containing M13mp18 DNA (10 nM), staple DNA strands (4 equiv), 20 mM Tris-HCl (pH 7.6), 12.5 mM MgCl$_2$, 1 mM EDTA, and 50 mM KCl from 85 to 15 °C at a rate of −1.0 °C/min. The DNA strands containing a divided GQ or i-motif sequence for introduction to the top side (a–b connector) and the bottom side (c–d connector) were also prepared using the same conditions as those of the DNA frame. The preassembled strands (5 equiv) were incorporated into the DNA frame by reannealing the solution from 40 (kept for 10 min) to 15 °C at a rate of −0.5 °C/min. For the GQ formation, the sample (2 μL) was added to the observation buffer containing 50 mM KCl. In the case of the i-motif formation, the sample (2 μL) was added to the pH 5.5 observation buffer.

The buffer conditions for the AFM observation are as follows: neutral condition with K^+ buffer: 10 mM Tris (pH 7.6), 12.5 mM MgCl$_2$, 50 mM KCl; acidic condition buffer: 10 mM MES (pH 5.5), 12.5 mM MgCl$_2$; acidic condition with K^+ buffer: 10 mM MES (pH 5.5), 12.5 mM MgCl$_2$, 50 mM KCl.

Preparation of the GQ/i-Motif Double-Stranded DNA in the DNA Frame. The DNA strands containing the GQ sequence for the top side and that containing the i-motif sequence for the bottom side were also prepared using the same conditions as those of the DNA frame. First, introduction of the i-motif-containing strand (5 equiv) covered with the toehold-protecting sequence into the DNA frame was carried out by reannealing the solution from 40 (kept for 10 min) to 15 °C at a rate of −0.5 °C/min. Then the GQ-containing strand (5 equiv) was introduced into the top side of the DNA frame (a–b connector) with the annealing step from 30 (stay for 6 min) to 12 °C at a rate of −0.4 °C/min. After that, the two-strands-attached DNA frame was purified by a Sephacyl S-400 gel-filtration column. The sequences for incorporated strands are described in the Supporting Information. For the preparation of the GQ/i-motif dsDNA, we first removed the toehold-protecting sequence in the bottom side by adding the release strand containing the complement to the toehold-protecting sequence (10 equiv). Then the samples were desalted twice by a centrifugal filter (10 K NMWL, Millipore Amicon) at rt for 10 min with the buffer that contained 10 mM Tris-HCl (pH 7.6), 1 mM EDTA, and 12.5 mM MgCl$_2$, and the samples were incubated at rt for 1 h. The desalted sample (2 μL) was added to 28 μL of the buffer for AFM observation and incubated at rt for 1 h. The states of DNA strands in the DNA frames in all the steps were checked by AFM observation.

AFM Imaging. Imaging was performed using a high-speed AFM (Nano Live Vision, RIBM, Tsukuba, Japan). The sample (2 μL) was deposited onto freshly cleaved mica, kept for 5 min, and then rinsed with the buffer. The sample was imaged in the observation buffer solution at rt using a small cantilever (BL-AC10EGS, Olympus Corporation, Tokyo, Japan) with a spring constant of 0.1−0.2 N/m with a resonant frequency of 400−1000 kHz in water. The AFM images were obtained at a

scan rate of 0.2−1.0 frame per second (fps). The data were collected by counting 50 to 150 DNA frames from the AFM images for each experiment.

Conflict of Interest: The authors declare no competing financial interest.

Supporting Information Available: The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsnano.5b03413.

DNA sequences, additional experiments, and additional AFM images (PDF)
Movie 2. High-speed AFM movie of the hybridization of the GQ-/i-motif-forming ssDNA by removal of K^+ (AVI)
Movie 3. High-speed AFM movie of the dissociation of the GQ/i-motif duplex by incubation with K^+ at pH 7.6 (AVI)
Movie 4. High-speed AFM movie of the dissociation of the GQ/i-motif duplex by incubation at pH 5.5 (AVI)
Movie 5. High-speed AFM movie of the dissociation of the GQ/i-motif duplex by incubation with K^+ at pH 5.5 (AVI)

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