ARTICLE

Received 26 Aug 2014 | Accepted 3 Feb 2015 | Published 7 Apr 2015

DOI: 10.1038/ncomms7640

Metal ion-directed dynamic splicing of DNA through global conformational change by intramolecular complexation

Toshihiro Ihara^{1,2}, Hiroyuki Ohura¹, Chisato Shirahama¹, Tomohiro Furuzono¹, Hiroshi Shimada¹, Hiroataka Matsuura¹ & Yusuke Kitamura^{1,2}

Chemically engineered DNAs—in which global conformation can be modulated in response to specific stimuli—could be allosteric functional DNAs themselves or work as a modulator of the functional nucleic acids such as DNAzymes and aptamers. Here, we show that two terpyridines built in the DNA backbone form a stable intramolecular 1:2 complex, $[M(\text{terpy})_2]^{2+}$, with divalent transition metal ions. Upon complexation, the DNA conjugates adopt a Ω -shape structure, in which two distal sequences located outside the terpyridines connect with each other to form a continuous segment with a specific structure or sequence. Such a DNA structure is globally controlled by local metal complexation events that can be rationally designed based on general coordination chemistry. This method is regarded as metal ion-directed dynamic sequence edition or DNA splicing. DNAzymes with peroxidase-like activity can thus be regulated by several transition metal ions through sequence edition techniques based on the Ω -motif.

¹Department of Applied Chemistry and Biochemistry, Graduate School of Science and Technology, Kumamoto University, 2-39-1 Kurokami, Chuo-ku, Kumamoto 860-8555, Japan. ²CREST, Japan Science and Technology Agency, 7 Gobancho, Chiyoda-ku, Tokyo 102-0076, Japan. Correspondence and requests for materials should be addressed to T.I. email: (toshi@chem.kumamoto-u.ac.jp).

Recently, several research groups have reported on the fabrication of specific DNA structures using metal ion-directed techniques^{1–21}. For example, two thymines and cytosines coordinate to Hg^{2+} and Ag^+ , respectively, to form a M-DNA (metal ion DNA complex) duplex through the formation of metal-centred nucleobase dimers^{7,8,12–15}. A silver ion can be accommodated in the nucleobase triplet CGC of a DNA triplex by substitution of the N3 proton of cytosine on the third strand, which stabilizes the triplex structure even under neutral pH^{10,11}. The knowledge of the specific coordination of nucleobases to these soft metal ions enabled us to prepare stable duplexes or triplexes by designing the sequences. On the other hand, several DNA analogues carrying metal chelators instead of natural nucleobases have been reported. Chelators, such as catechol, phenylenediamine, aminophenol and hydroxypyridone, selectively coordinate to certain transition metal ions to form stable M-DNA duplexes or triplexes^{4–6}. The DNA conjugates with chelator molecules on their backbone have also been reported to form unique structures^{9,16–21}.

In the past decade, a number of functional nucleic acids, such as ribozymes, DNazymes and aptamers, have been isolated using techniques of molecular evolution^{22,23}. These nucleic acids have been applied to analytical systems for various biomolecules as functional modules for analyte recognition and signal amplification^{24–27}. Prescribed higher-ordered structures are critically important for these nucleic acids to express their intrinsic functions. Therefore, techniques that control formation of their structures should be useful to modulate their functions. If the chemical groups, which respond to certain stimuli by changing their structure, are incorporated into the backbone of the functional DNAs or their complementary sequences, we could regulate their functions by controlling conformational changes in the DNAs. This strategy is general in the sense that it targets the higher-ordered structure of functional nucleic acids rather than each of their particular functions themselves. The techniques for drastic changes in DNA conformation could also be applied to regulate gene expression by certain stimuli such as decoy, antisense and RNAi strategies.

Herein, we synthesized 2,2':6',2''-terpyridine (terpy)-based amidite reagents. Terpy units can be incorporated into any positions in the DNA backbone with these amidite reagents using automated DNA synthesis. Terpy forms a stable complex with Fe^{2+} and some other divalent transition metal ions^{28,29}. We expected that the stiffness and conformation of the DNA backbone could be controlled through complexation with certain metal ions. Then, it would enable us to regulate the activity of functional nucleic acids. We have already reported the effect of metal ions on the stability of DNA structures consisting of the conjugates with one terpy (synthesized using **1** mentioned below). The terpy incorporated in the stem moiety of the duplex stabilized the structure by complexation, which confers entropic benefit on the DNA. In contrast, the structure of bimolecular triplexes carrying a terpy unit in its loop moiety significantly destabilizes on complexation with divalent transition metal ions³⁰. This can be explained by the change in the length of the terpy unit in the loop of the triplex by metal coordination; the metal-bound terpy connecting two pyrimidine strands is too short to cap the base triplet in the structure of the DNA triplex.

In the present study, two terpy units are incorporated into the backbone of a DNA strand to form terpy₂-DNA conjugates, in which two terpys are located at distant positions from each other with an intervening sequence. It is expected that transition metal ions, such as Fe^{2+} , forms a 1:2 complex with the two terpys in a terpy₂-DNA conjugate. To form such an intramolecular complex, the backbone of terpy₂-DNA conjugates make adopt an Ω -shaped conformation, where the two distal sequences outside

both terpys in the sequence are directly connected with each other to form a continuous stretch of the specific structure or sequence. Such global conformational control of DNA would be regarded as metal ion-directed sequence edition or reversible splicing of DNA. We perform allosteric regulation of catalytic activity of DNzyme through metal ion-directed sequence edition of DNA.

Results

DNA preparation and system design. In this study, we aimed to regulate the function of DNzyme with peroxidase-like activity^{31,32} by conformational control of the terpy₂-DNA conjugate. To incorporate terpy units into the backbone of the DNA, terpy-based amidite reagents were prepared. We synthesized two amidite reagents **1** and **2** (Fig. 1a) according to the schemes shown in Supplementary Information. Although both of them have a 2,2':6',2''-terpyridine structure with two ethylene linker chains connecting to phosphoramidite and dimethoxytrityl-protected hydroxyl groups, the substitution position of the linkers are different. **1** and **2** tether the two linker chains from 6,6''- and 5,5''-positions of terpy, respectively.

The sequences of the DNAs used in this study are shown in Fig. 1b. The four terpy₂-DNA conjugates, **tp₂hp1**, **tp₂hp2**, **tp₂tmp1** and **tp₂tmp2**, were prepared using **1** and **2** according to the standard phosphoramidite method shown in Supplementary Information. The last letter in the names of the terpy₂-DNA conjugates, '1' or '2', stands for the amidite reagents **1** and **2** built in their sequences, respectively. We designed two systems for regulation of oxidation reactions by DNzyme. In one system, we used the modified DNzyme, **tp₂hp1** and **tp₂hp2**, which can adopt a hairpin form by intramolecular metal complexation. The sequence of DNzyme was split into two parts³³, and each half was attached to both ends of a part of the complementary sequence of the H-ras gene³⁴ through terpys to form **tp₂hp1** and **tp₂hp2**. The catalytic activity of **tp₂hp1** and **tp₂hp2** were expected to be restored when forming a hairpin conformation induced by intramolecular 1:2 metal complexation (Fig. 1c). Reconstruction of an integrated active DNzyme on the allosteric template, **tp₂tmp1** and **tp₂tmp2**, was expected in another system. The same DNzyme mentioned above was also split into two parts, and then the short binding arms were attached to the end of these parts to form a pair of the half DNzymes³³, **zymR** and **zymL**. The outside two sequences in **tp₂tmp1** and **tp₂tmp2** were designed to be complementary to the binding arms of **zymR** and **zymL**. Therefore, **tp₂tmp1** and **tp₂tmp2** are expected to work as an effective template when both outside sequences come in proximity with each other by forming an Ω -shaped conformation through 1:2-type intramolecular metal complexation (Fig. 1d). The loop sequences between the two terpys of **tp₂tmp1** and **tp₂tmp2** are designed to be complementary to miR-34 (microRNA)³⁵.

Preliminary studies of complexation. Complexation of the terpy₂-DNA conjugates, **tp₂tmp1** and **tp₂tmp2**, with metal ions were investigated by ultraviolet-visible (vis) titrations. Four divalent transition metal ions, Fe^{2+} , Ni^{2+} , Cu^{2+} and Zn^{2+} , were used in this study (Fig. 2). The aliquots of transition metal ions were added into the conjugate solutions, and their ultraviolet-vis spectrum was recorded after each addition. The spectral changes and the plots of absorbance around the peak with Fe^{2+} titration are shown in Fig. 2a,b. The complexation behaviour of the two conjugates was completely different. **tp₂tmp1** showed stoichiometric complexation with Fe^{2+} . The absorbance increased monotonously and saturated at the point of $[\text{Fe}^{2+}]/[\text{tp}_2\text{tmp1}] = 2.0$ (Fig. 2a), indicating that each of the two terpys on a **tp₂tmp1** bound a Fe^{2+} to form two $[\text{Fe}(\text{terpy})]^{2+}$ on

a **tp₂tmp1**. In contrast, the stoichiometry of **tp₂tmp2** complexation was clearly shown to be 1.0 ($= [\text{Fe}^{2+}]/[\text{tp}_2\text{tmp2}]$) (Fig. 2b), indicating that the terpy units formed $[\text{Fe}(\text{terpy})_2]^{2+}$ on **tp₂tmp2**. Further change was not observed in the spectra even with more additional Fe^{2+} . The characteristic band was observed at 530 nm. This is attributed to the metal-to-ligand charge-transfer band of the 1:2 complex, $[\text{Fe}(\text{terpy})_2]^{2+}$ (refs 28,29). Interestingly, this meant that the complex $[\text{Fe}(\text{terpy})_2]^{2+}$ did not form on **tp₂tmp1** but exclusively formed on **tp₂tmp2**. All of the results observed in the ultraviolet–vis titrations were reproduced in the presence of the complementary DNA, **ctmp**, which is the tandem sequence complementary to the two distal sequences outside of the two terpys.

The only difference between **tp₂tmp1** and **tp₂tmp2** is the tethering positions of the terpys connected to DNA backbones. We calculated the structures of both complexes using the density functional theory (DFT) method (B3LYP/6-31G⁺). The optimized structures of $[\text{Fe}(\text{terpy})_2]^{2+}$ are shown in Fig. 3. The structure of Fig. 3a is the complex consisting of a Fe^{2+} and two 6,6''-tethered terpys and Fig. 3b is of a Fe^{2+} and two 5,5''-tethered terpys, each of which is the complex expected to form on **tp₂tmp1** (or **tp₂tmp1/ctmp**) and **tp₂tmp2** (or **tp₂tmp2/ctmp**), respectively. In the structure of $[\text{Fe}(\text{terpy})_2]^{2+}$ with 6,6''-tethered terpys, the linker chains on a terpy almost touches the central pyridine ring of another terpy in the complex. This steric hindrance should make the complex unstable or impossible to form. In contrast, this hindrance was not observed in the complex consisting of 5,5''-tethered terpys. This would explain the difference in complexation behaviour between **tp₂tmp1** and **tp₂tmp2** observed in ultraviolet–vis titration.

The stoichiometry of Ni^{2+} –**tp₂tmp2** complexation was almost the same with Fe^{2+} mentioned above (Fig. 2c, spectral change: see Supplementary Fig. 1). That is, regardless of the feeding ratio, $[\text{M}^{2+}]/[\text{tp}_2\text{tmp2}]$, Fe^{2+} and Ni^{2+} seem to form a rather stable complex, $[\text{M}(\text{terpy})_2]^{2+}$, on **tp₂tmp2**. Meanwhile, Cu^{2+} (Fig. 2d, spectral change: see Supplementary Fig. 2) and Zn^{2+} (see Supplementary Fig. 3) showed a multiphasic unique spectral change. For both titrations, two apparent inflection points were observed at $[\text{M}^{2+}]/[\text{tp}_2\text{tmp2}]$ ratios of 1.0 and 2.0, indicating that the two types of complexes with different stoichiometry were successively formed with the addition of the metal ions. 1:2 complex, $[\text{M}(\text{terpy})_2]^{2+}$, formed on **tp₂tmp2** at a lower feeding ratio, which changed to a 1:1 complex, $[\text{M}(\text{terpy})]^{2+}$ upon further addition of metal ions. The difference in the stoichiometry of the complexes of the four transition metal ions could be explained by the relative magnitude of successive binding constants, K_1 and K_2 ; Fe^{2+} : $\log K_1 = 7.1$, $\log K_2 = 13.8$; Ni^{2+} : 10.7, 11.2; Zn^{2+} : 6.7, 5.2; Cu^{2+} : 13.0, not available (that is, K_2 is much smaller than K_1). Whereas the magnitudes of K_1 are larger than K_2 for Fe^{2+} and Ni^{2+} , the opposite is true for Cu^{2+} and Zn^{2+} (refs 28,36).

To identify the complex, an equimolar mixture of terpy₂–DNA and Fe^{2+} was subjected to matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. The short terpy₂–DNA conjugate (18-mer DNA conjugate carrying two terpy units) was prepared for this study using the terpy amidite 2. Using the conjugate with a lower molecular weight, we can obtain accurate and precise m/z values of the complexes, even for multimeric DNAs. The result are shown in Supplementary Fig. 4. The only complex observed was $[\text{terpy}_2\text{–DNA} + 54]^-$ for the

Figure 1 | DNA structures, sequences and constructions of allosteric DNAzymes. (a) Structures of terpyridine-based amidite reagents, **1** and **2**. **(b)** Sequences of terpy₂–DNA conjugates and DNAs used in this study. **(c)** Working principle of metal ion-dependent DNAzyme, **tp₂hp1** and **tp₂hp2**. The modified DNAzyme adopts an active hairpin structure by intramolecular metal complexation. **(d)** Allosteric regulation of split DNAzyme activity by metal ion-directed dynamic sequence edition of the template, **tp₂tmp1** and **tp₂tmp2**.

Figure 2 | Preliminary spectrophotometric studies for metal complexation of $\text{tp}_2\text{tmp1}$ and $\text{tp}_2\text{tmp2}$ with divalent transition metal ions.

(a) Spectral change of $\text{tp}_2\text{hp1}$ in ultraviolet-vis titration of Fe^{2+} . Inset: the absorbance at 356 nm was plotted against the feeding ratio, $[\text{Fe}^{2+}]/[\text{tp}_2\text{hp1}]$. (b) Spectral change of $\text{tp}_2\text{hp2}$ in ultraviolet-vis titration of Fe^{2+} . Inset: the absorbance at 337 nm was plotted against the feeding ratio, $[\text{Fe}^{2+}]/[\text{tp}_2\text{hp2}]$. (c) The result of Ni^{2+} titration. The absorbance at 336 nm was plotted against the feeding ratio, $[\text{Ni}^{2+}]/[\text{tp}_2\text{tmp2}]$. (d) The result of Cu^{2+} titration. The absorbance at 339 nm was plotted against the feeding ratio, $[\text{Cu}^{2+}]/[\text{tp}_2\text{tmp2}]$. The titrations were performed with $2.0 \mu\text{M}$ DNA in HEPES buffer solution (10 mM, pH 7.0) containing 500 mM NaCl at 25°C .

Figure 3 | Optimized structures of $[\text{Fe}(\text{terpy})_2]^{2+}$ with 6,6''-substituted and 5,5''-substituted terpyridines. Both structures were optimized with DFT calculation (B3LYP/6-31G*).

(a) Optimized structure of $[\text{Fe}(\text{terpy})_2]^{2+}$ with 6,6''-terpyridines, which is proposed to form on $\text{tp}_2\text{hp1}$ and $\text{tp}_2\text{tmp1}$. (b) Optimized structure of $[\text{Fe}(\text{terpy})_2]^{2+}$ with 5,5''-terpyridines, which is proposed to form on $\text{tp}_2\text{hp2}$ and $\text{tp}_2\text{tmp2}$. The two structures shown in the centre and the right-hand side are different representations of the same structures, stick model and Van der Waals surface, respectively.

equimolar mixture. The m/z of 54 corresponds to the mass difference between Fe^{2+} and two protons. No multimeric DNA complexes were observed. This result shows that the conjugate, terpy₂-DNA, does not form intermolecular multimeric complexes, but intramolecularly forms the complex $[\text{Fe}(\text{terpy})_2]^{2+}$.

Metallo-regulation of thermal stability of the duplexes. The melting behaviour was studied for the duplexes consisting of the terpy₂-DNA conjugates and the complementary DNA, **ctmp**. The ultraviolet melting curves of the duplexes, **tp₂ttmp1/ctmp** and **tp₂ttmp2/ctmp**, recorded in the presence of the divalent transition metal ions are shown in Fig. 4. The melting curves of **tp₂ttmp1/ctmp** were scarcely affected by any transition metal ions (Fig. 4a). In contrast, the duplex structure of **tp₂ttmp2/ctmp** was apparently stabilized by the addition of an equimolar amount of transition metal ions (Fig. 4b). In addition, the metal ions appeared to change the shape of the melting curves of **tp₂ttmp2/ctmp** to be more cooperative, indicating that the two sequences outside of the terpy₂ cooperatively dissociated in a narrow temperature range in the presence of the metal ions.

The highly cooperative melting behaviour also suggests that $[\text{Fe}(\text{terpy})_2]^{2+}$ observed in ultraviolet-vis titration for **tp₂ttmp2** was an intramolecular complex formed on **tp₂ttmp2•Fe²⁺**. That is, **tp₂ttmp2** formed an Ω -shaped conformation through intramolecular 1:2 complexation and, concomitantly, the distal two segments located outside of two terpy₂ came close in proximity

with each other to form a continuous stretch of the complementary sequence for **ctmp** (Fig. 4c). The duplexes would be stabilized by entropic benefit through metal ion-directed global conformational change of DNA. This could be regarded as dynamic sequence edition. Meanwhile, the complex formation does not affect the global conformation of **tp₂ttmp1**, because each of the terpy₂ binds Fe^{2+} independently, which is the reason the metal ions scarcely stabilize the duplex **tp₂ttmp1/ctmp**.

The melting temperature of **ctmp/ctmp** was 38 °C under the same conditions. Although the Ω -shaped conformation of **tp₂ttmp2•Fe²⁺** gave the same sequence with **ctmp**, the duplex stability of **tp₂ttmp2•Fe²⁺/ctmp** did not come up to **ctmp/ctmp**. The structure of **tp₂ttmp2•Fe²⁺** could be improved for its structural continuity. Comparable results were commonly observed for chemical ligation products with non-natural structures at the connecting points³⁷. It could be improved by optimization of the linker chain structure of terpy units in terpy₂-DNA.

At the point of equimolar ratio ($[\text{M}^{2+}]/[\text{tp}_2\text{ttmp2}] = 1$), all transition metal ions used in the melting study stabilized the duplex **tp₂ttmp2/ctmp** to a similar extent. However, the dependences of the stabilization effects on the feeding ratio were different for each of the metal ions. For Fe^{2+} and Ni^{2+} , the duplex remained stable at $[\text{M}^{2+}]/[\text{tp}_2\text{ttmp2}] = 2$ and 3. In contrast, the duplex was destabilized at higher feeding ratios for Cu^{2+} and Zn^{2+} (Fig. 4d). The results essentially coincide with those obtained in the ultraviolet-vis titration. The duplex stability of **tp₂ttmp2/ctmp** was maintained even in the presence of the excess amount of Fe^{2+} and Ni^{2+} , because the Ω -shaped conformation of **tp₂ttmp2** would be preserved due to the binding properties of the metal ions with terpy, $K_1 < K_2$. Meanwhile, for Cu^{2+} and Zn^{2+} , the global conformation of **tp₂ttmp2** would be changed from Ω - to a linear-form accompanying the transition of the complex types formed on **tp₂ttmp2** from $[\text{M}(\text{terpy})_2]^{2+}$ (on **tp₂ttmp2•M²⁺**) to $2[\text{M}(\text{terpy})]^{2+}$ (on **tp₂ttmp2•2M²⁺**) with increasing amounts of the ions due to their magnitudes of the two successive binding constants with terpy, $K_1 > K_2$ (ref. 36). Especially, the binding of Cu^{2+} with endocyclic nitrogens of nucleobases is strong^{38,39}. This would explain the significant inhibition of the duplex formation by Cu^{2+} at higher $[\text{Cu}^{2+}]/[\text{tp}_2\text{ttmp2}]$ ratios.

Allosteric regulation of DNAzyme activity. We constructed the two systems to regulate the activity of DNAzyme by specific coordination of metal ions. The active form of the DNAzyme is a G-quadruplex (G4) structure that requires a hemin as a cofactor^{31,32}. The catalytic activity was monitored by oxidation of ABTS (2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), which shows green in colour after oxidation⁴⁰.

Figure 5a shows the time course of the reaction profiles for the conjugates, **tp₂hp1** or **tp₂hp2**, in the presence of an equivalent amount of Fe^{2+} . The activity of **tp₂hp2** was apparently restored by Fe^{2+} . Such activation was not observed for **tp₂hp1**. As expected from the preliminary studies mentioned above, only **tp₂hp2** seemed to adopt a hairpin structure by intramolecular $[\text{Fe}(\text{terpy})_2]^{2+}$ formation and restored the active form of DNAzyme (Fig. 1c). The conjugate **tp₂hp2** could be regarded as a semi-artificial metallo-DNAzyme. However, the activity level of **tp₂hp2•Fe²⁺** was very little compared with the system of **tp₂ttmp2**. The G4-structure formed on **tp₂hp2•Fe²⁺** might not be ideal as a catalyst. This could be improved by fine-tuning of the structures around terpy₂.

Another allosteric system was also tested to control the activity of DNAzyme. Here **tp₂ttmp2** was used as the tunable template to activate the split DNAzyme (**zymR** and **zymL**) as shown in

Figure 4 | Effects of the transition metal ions on melting behaviour of the duplex, terpy₂-DNA/ctmp. (a) Ultraviolet melting curves of **tp₂ttmp1/ctmp** in the presence of divalent transition metal ions. The dotted curve was obtained in the absence of any transition metals. Red, green, black and blue curves show the melting curves obtained in the presence of Fe^{2+} , Cu^{2+} , Zn^{2+} and Ni^{2+} , respectively. (b) Ultraviolet melting curves of **tp₂ttmp2/ctmp** in the presence of divalent transition metal ions. Assignment of the curves are the same with a. Melting experiments were carried out for 1.0 μM DNAs and the metal ions in HEPES buffer solution (10 mM, pH 7.0). (c) One of the possible structures of the Ω -shape DNA duplex, **tp₂ttmp2•Fe²⁺/ctmp**. The model was geometry-optimized by AMBER* force field with GB/SA (generalized Born/surface area) solvent model using MacroModel version 9.5. (d) The effect of the feeding ratio of the metal ions on the melting temperatures of the duplex, **tp₂ttmp2/ctmp**.

Figure 5 | Metal ion-directed regulation of DNAzyme activity. (a) Time courses of the ABTS oxidation by **tp₂hp1** and **tp₂hp2** in the presence of Fe²⁺. The dotted curve was obtained without the conjugates in the absence of any transition metals. Solid curves show the reaction profiles obtained in the presence of the DNA conjugates. Blue: **tp₂hp1** + Fe²⁺; red: **tp₂hp2** + Fe²⁺; black: **tp₂hp2**, no metal ions. (b) Time courses of the ABTS oxidation by the split DNAzyme with **tp₂tmp2** in the presence of Fe²⁺ and Ni²⁺ and the effects of reaction inhibitors. Red: **zymR/zymL/tp₂tmp2** + Fe²⁺; blue: **zymR/zymL/tp₂tmp2** + Ni²⁺; black: **zymR/zymL/tp₂tmp2**, no metal ions; red dotted: **zymR/zymL/tp₂tmp2** + Fe²⁺ + EDTA; red broken: **zymR/zymL/tp₂tmp2** + Fe²⁺ + cDNA to the loop region (**miR**). Typical reaction profiles are shown in **a** and **b**. The errors in repetitive reactions were within 10% (s.d., $n = 3$). DNA complexes (all DNA components: **zymR**, **zymL**, **tp₂hp1**, **tp₂hp2**, **tp₂tmp1** and **tp₂tmp2**), 0.40 μ M; hemin, 2.0 μ M; MCl₂, 0.40 μ M; H₂O₂, 2.0 mM; ABTS, 100 μ M; HEPES, 14 mM (pH 7.0); NaCl, 700 mM; KCl, 140 mM. (c) Images of reaction solutions shown in **b**. (d) Differential circular dichroism (CD) spectra of the ternary complex, **zymR/zymL/tp₂tmp2**. Red: (**zymR/zymL/tp₂tmp2** + Fe²⁺) – (**tp₂tmp2/ctmp** + Fe²⁺), black: (**zymR/zymL/tp₂tmp2**) – (**tp₂tmp2/ctmp**). CD spectra were measured for 3.0 μ M DNAs and the metal ions in HEPES buffer solution (20 mM, pH 7.0) containing 1,000 mM NaCl, 200 mM KCl at 15 °C.

Fig. 1d. The time course of the reaction profiles is shown in Fig. 5b. Equivalent concentrations of Fe²⁺ and Ni²⁺ restored the activity of the split DNAzyme in the presence of **tp₂tmp2**. The effect of metal ions could be observed by naked eyes (Fig. 5c). The regained activity level was comparable to that of the positive control, **zymR/zymL/czym** (see Supplementary Fig. 6). Cu²⁺ and Zn²⁺ also showed moderate effect in restoration of split DNAzyme activity (see Supplementary Fig. 5). As we expected, global conformation of **tp₂tmp2** was fixed to a Ω -shape by intramolecular formation of [M(terpy)₂]²⁺. Then, **tp₂tmp2**•M²⁺ worked as an effective template to reconstruct the integrated active form of DNAzyme.

The activity was almost completely suppressed by a large excess amount of EDTA and an equimolar amount of the single-stranded 23-mer DNA (**miR**) with an miR-34 sequence, which is complementary to the loop sequence (Fig. 5b). The results of these inhibition experiments confirmed our scheme for the regulation of DNAzyme activity. EDTA removed Fe²⁺ from the complex as a masking reagent. The single-stranded DNA with the miR-34 sequence straightened the loop region of **tp₂tmp2** by hybridization and made it impossible to form [Fe(terpy)₂]²⁺.

The ternary DNA complex **zymR/zymL/tp₂tmp2** was subjected to circular dichroism (CD) spectroscopy. Figure 5d shows the differential CD spectra. To show only the contribution of the DNAzyme moiety, the CD spectrum of **tp₂tmp2/ctmp** (duplex + loop) was subtracted from those of **zymR/zymL/tp₂tmp2**

(reconstituted DNAzyme + duplex + loop) obtained in the absence and in the presence of Fe²⁺. The characteristic CD band for the mixed-type G4-structure (or the mixture of parallel and antiparallel folded species) was induced by the addition of Fe²⁺ (refs 41,42). This suggests that Fe²⁺ activated the DNAzyme by induction of a valid higher-ordered structure through sequence edition of the template, **tp₂tmp2**.

Discussion

Our aim in this study was to induce a drastic conformational change of global DNA (secondary) structure and its application for the allosteric regulation of the function of nucleic acids by certain stimuli. Although several stimuli-responsive DNA conjugates that change their local conformation have been reported^{43–45}, the systems of allosteric control of the global DNA structures are quite limited^{9,17,46,47}. Even more, as far as we know, there is no precedent of conformational modulation of DNA aiming at sequence edition. The formation of a Ω -shape structure by intramolecular interaction between two specific points on a sequence could be regarded as stimuli-responsive artificial splicing. That is, the distal two segments are directly connected with each other by clipping out the intervening loop sequence.

In this study, the specific coordination of terpy to transition metal ions was chosen as a connecting reaction to show the

availability of a Ω -motif for regulation of DNA function. Actually, while the phosphates in DNA backbone and nucleobases can coordinate to the transition metal ions³⁹, the ions, especially Fe^{2+} and Ni^{2+} , selectively bind two terpy to form a Ω -shaped structure under experimental conditions. The common preference in coordination chemistry of terpy was retained even for complexation on DNA. Therefore, the Ω -shape structure formation could be designed based on general knowledge in coordination chemistry. The kind and concentration of the metal ions, the structure of the terpy and the design of the conjugate architecture could be effective factors to control the system. Especially, it was interesting that local structural differences (just as $\approx 1.4 \text{ \AA}$) in terpy units, such as substitution positions, were critically important to define the global structure.

We succeeded in the regulation of DNAzyme activity using the Ω -motif. Judging from the results of ultraviolet–vis titration, ultraviolet melting, CD spectroscopy, modelling analysis by DFT and ABTS oxidation and inhibition experiments, the activity of DNAzyme was regulated basically according to the proposed scheme shown in Fig. 1. However, the difference in melting temperatures of the duplex **tp₂t_{mp}2/ctmp** observed in the presence and absence of Fe^{2+} might not be enough to solely explain the sharp response in restoration of DNAzyme activity. At least, the CD spectra showed that Fe^{2+} induced the active G4-structure of DNAzyme. Fe^{2+} could work as an essential factor not only to assemble the split DNAzymes but also to induce the active G4-structure through complexation.

To construct the system for artificial (non-enzymatic or chemical) splicing of DNA, we can choose appropriate clipping reactions from many bioorthogonal chemistries including click reactions^{48,49} and photochemical reactions^{37,44–46,50}. The techniques could be applied for signal amplification in bioanalyses through DNA circuits based on autonomous succession of strand exchange^{26,51}. The regulation of cell function through the control of gene expression would also be very compatible and a promising application.

Methods

Synthesis. Preparation³⁰ of the amidite reagents, **1** and **2**, and DNAs are described in the Supplementary Information.

Ultraviolet–vis titration. The aliquots (typically, $0.40 \mu\text{l}$)^{16,52,53} of a 0.50 mM stock solution of MCl_2 ($\text{M} = \text{Fe}^{2+}$, Ni^{2+} , Cu^{2+} or Zn^{2+}) were titrated into a 1.0 ml solution of $2.0 \mu\text{M}$ DNA or DNA complex (**tp₂t_{mp}1**, **tp₂t_{mp}2**, **tp₂t_{mp}1/ctmp** or **tp₂t_{mp}2/ctmp**, in 10 mM HEPES (pH 7.0), 500 mM NaCl) in a cuvette, and then the solution was mixed well. The ultraviolet–vis spectra were measured using a spectrophotometer V-650 (Jasco) at 25°C , after 30 min incubation following each addition.

Ultraviolet melting. Thermal denaturation experiments were performed^{9,54,55} in HEPES buffer solution (10 mM , pH 7.0) on a UV-1650 (Shimadzu) ultraviolet–vis spectrophotometer equipped with a Peltier thermal controller. Concentrations of each DNA strand and MCl_2 ($\text{M} = \text{Fe}^{2+}$, Ni^{2+} , Cu^{2+} or Zn^{2+}) were $1.0 \mu\text{M}$. Prior to the beginning of each melt, the samples were degassed at 85°C for 5 min and then annealed by slowly cooling to 0°C . In the denaturation experiments, the solutions were heated at a rate of 0.5 deg min^{-1} after equilibration for 20 min at 0°C .

The temperature that gave the maximum first derivative of the melting curve was used as the melting temperature, a measure of the thermal stability of the duplexes.

Circular dichroism measurement. CD^{56,57} spectra were measured in HEPES buffer solution (20 mM , pH 7.0) containing 1.0 M NaCl and 200 mM KCl on a J-725 Spectropolarimeter (Jasco) equipped with a Peltier thermal controller, at 15°C . Concentrations of all DNA strands and FeCl_2 were $3.0 \mu\text{M}$. Prior to the beginning of each melt, the samples were degassed at 85°C for 5 min and then annealed by slowly cooling to 15°C .

To only show the contribution of the DNAzyme moiety, the CD spectrum of **tp₂t_{mp}2/ctmp** (duplex + loop) was subtracted from those of **zymR/zymL/tp₂t_{mp}2**

(reconstituted DNAzyme + duplex + loop) obtained in the absence and in the presence of Fe^{2+} .

Molecular modelling. Both complex ($[\text{Fe}(\text{terpy})_2]^{2+}$) structures were calculated by the DFT method (B3LYP/6-31G*) using Spartan 10 (Wavefunction Inc., USA). The molecular models shown in Fig. 4c were constructed with MacroModel (ver. 9.5, Schrödinger Inc., USA). The initial structure was built as a standard double helix using the Maestro (Schrödinger Inc.) fragment library. The optimization was performed with an AMBER* force field with generalized Born/surface area treatment of solvation.

Allosteric catalytic reactions by split DNAzymes. Into the mixed solution of DNA and hemin,^{40,58,59} was added the solution containing MCl_2 ($\text{M} = \text{Fe}^{2+}$, Ni^{2+} , Cu^{2+} or Zn^{2+}), H_2O_2 and ABTS to prepare 1.0 ml of the reaction solution in a cuvette. The final concentrations of each of the components were as follows: DNA complexes (all DNA components: **zymR**, **zymL**, **zym**, **tp₂hp1**, **tp₂hp2**, **tp₂t_{mp}1** and **tp₂t_{mp}2**), $0.40 \mu\text{M}$; hemin, $2.0 \mu\text{M}$; MCl_2 , $0.40 \mu\text{M}$; H_2O_2 , 2.0 mM ; ABTS, $100 \mu\text{M}$; HEPES, 14 mM (pH 7.0); NaCl, 700 mM ; KCl, 140 mM . The progress of ABTS oxidation was monitored at 15°C by the change in absorbance at 414 nm . The time courses of absorbance change were monitored using a spectrophotometer V-650 (Jasco) equipped with a Peltier thermal controller.

References

- Clever, G. H., Kaul, C. & Carell, T. DNA–metal base pair. *Angew. Chem. Int. Ed.* **46**, 6226–6236 (2007).
- Scharf, P. & Müller, J. Nucleic acids with metal-mediated base pairs and their applications. *ChemPlusChem* **78**, 20–34 (2013).
- Biver, T. Stabilisation of non-canonical structures of nucleic acids by metal ions and small molecules. *Coord. Chem. Rev.* **257**, 2765–2783 (2013).
- Tanaka, K. et al. Programmable self-assembly of metal ions inside artificial DNA duplex. *Nat. Nanotechnol.* **1**, 190–194 (2006).
- Tanaka, K., Tengeji, A., Kato, T., Toyama, N. & Shionoya, M. A discrete self-assembled metal array in artificial DNA. *Science* **299**, 1212–1213 (2003).
- Takezawa, Y., Maeda, W., Tanaka, K. & Shionoya, M. Discrete self-assembly of iron(III) ions inside triple-stranded artificial DNA. *Angew. Chem. Int. Ed.* **48**, 1081–1084 (2009).
- Ono, T. et al. Synthesis of covalently linked parallel and antiparallel DNA duplexes containing the metal-mediated base pairs T–Hg(II)–T and C–Ag(I)–C. *Chem. Commun.* **47**, 1542–1544 (2011).
- Ono, A., Torigoe, H., Tanaka, Y. & Okamoto, I. Binding of metal ions by pyrimidine base pair in DNA duplex. *Chem. Soc. Rev.* **40**, 5855–5866 (2011).
- Ihara, T., Takeda, Y. & Jyo, A. Metal ion-directed cooperative triple helix formation of glutamic acid–oligonucleotide conjugate. *J. Am. Chem. Soc.* **123**, 1772–1773 (2001).
- Ihara, T., Ishii, T., Araki, N., Wilson, A. W. & Jyo, A. Silver ion unusually stabilizes the structure of a parallel-motif DNA triplex. *J. Am. Chem. Soc.* **131**, 3826–3827 (2009).
- Urata, S., Miyahata, T., Matsuura, H., Kitamura, Y. & Ihara, T. Alteration of DNAzyme activity by silver ion. *Chem. Lett.* **43**, 1020–1022 (2014).
- Urata, H., Yamaguchi, E., Funai, T., Matsumura, Y. & Wada, S. Incorporation of thymine nucleotides by DNA polymerase through T–Hg^{II}–T base pairing. *Angew. Chem. Int. Ed.* **49**, 6516–6519 (2010).
- Park, K. S. & Park, H. G. Technological applications arising from the interaction of DNA bases with metal ions. *Curr. Opin. Biotechnol.* **28**, 17–24 (2014).
- Guo, W. et al. Reversible Ag⁺-crosslinked DNA hydrogels. *Chem. Commun.* **50**, 4065–4068 (2014).
- He, X. & Ma, N. A general strategy for label-free sensitive DNA detection based on quantum dot doping. *Anal. Chem.* **86**, 3676–3681 (2014).
- Shimada, H., Sakurai, T., Kitamura, Y., Matsuura, H. & Ihara, T. Metallo-regulation of the bimolecular triplex formation of peptide nucleic acid. *Dalton Trans.* **42**, 16006–16013 (2013).
- Göriz, M. & Krämer, R. Allosteric control of oligonucleotide hybridization by metal ion-induced cyclization. *J. Am. Chem. Soc.* **127**, 18016–18017 (2005).
- Graf, N., Göriz, M. & Krämer, R. A metal-ion-releasing probe for DNA detection by catalytic signal amplification. *Angew. Chem. Int. Ed.* **45**, 4013–4015 (2006).
- Choi, J. S. et al. Synthesis of DNA triangles with vertexes of bis(terpyridine)iron(II) complexes. *J. Am. Chem. Soc.* **126**, 8606–8607 (2004).
- Yang, H. & Sleiman, H. F. Templated synthesis of highly stable, electroactive, and dynamic metal-DNA branched junctions. *Angew. Chem. Int. Ed.* **47**, 2443–2446 (2008).
- Biankó, G. & Häner, R. A metal-coordinating DNA hairpin mimic. *ChemBiochem* **5**, 1063–1068 (2004).
- Ellington, A. D. & Szostak, J. W. In vitro selection of RNA molecules that bind specific ligands. *Nature* **346**, 818–822 (1990).
- Breaker, R. R. & Joyce, G. F. A DNA enzyme that cleaves RNA. *Chem. Biol.* **1**, 223–229 (1994).

24. Liu, J., Cao, Z. & Lu, Y. Functional nucleic acid sensors. *Chem. Rev.* **109**, 1948–1998 (2009).
25. Vinkenborg, J. L., Karnowski, N. & Famulok, M. Aptamers for allosteric regulation. *Nat. Chem. Biol.* **7**, 519–527 (2011).
26. Zhang, H., Li, F., Dever, B., Li, X.-F. & Le, X. C. DNA-mediated homogeneous binding assays for nucleic acids and proteins. *Chem. Rev.* **113**, 2812–2841 (2013).
27. Li, Y. & Lu, Y. (eds) *Functional Nucleic Acids for Analytical Applications* (Springer, 2009).
28. Schubert, U. S., Hofmeier, H. & Newkome, G. R. *Modern Terpyridine Chemistry* (Wiley, 2006).
29. Schwarz, G., Hasslauer, I. & Kurth, D. G. From terpyridine-based assemblies to metallo-supramolecular polyelectrolytes (MEPEs). *Adv. Colloid. Interface Sci.* **207**, 107–120 (2014).
30. Ihara, T., Sato, Y., Shimada, H. & Jyo, A. Metalloregulation of triple helix formation by control of the loop conformation. *Nucleos. Nucleot. Nucleic Acids* **27**, 1084–1096 (2008).
31. Li, Y. & Sen, D. Toward an efficient DNAzyme. *Biochemistry* **36**, 5589–5599 (1997).
32. Travascio, P., Li, Y. & Sen, D. DNA-enhanced peroxidase activity of a DNA aptamer-hemin complex. *Chem. Biol.* **5**, 505–517 (1998).
33. Kolpashchikov, D. M. Split DNA enzyme for visual single nucleotide polymorphism typing. *J. Am. Chem. Soc.* **130**, 2934–2935 (2008).
34. Reddy, E. P., Reynolds, R. K., Santos, E. & Barbacid, M. A point mutation is responsible for the acquisition of transforming properties by the T24 human bladder carcinoma oncogene. *Nature* **300**, 149–152 (1982).
35. Hau, A., Ceppi, P. & Peter, M. E. CD95 is part of a Let-7/p53/miR-34 regulatory network. *PLoS ONE* **7**, e49636 (2012).
36. Bjerrum, J., Schwarzenbach, G. & Sillén, L. G. *Compiled. Stability constants of Metal-Ion Complexes, with Solubility Products of Inorganic Substances* (The Chemical Society, 1957).
37. Ihara, T., Fujii, T., Mukae, M., Kitamura, Y. & Jyo, A. Photochemical ligation of DNA conjugates through anthracene cyclodimer formation and its fidelity to the template sequences. *J. Am. Chem. Soc.* **126**, 8880–8881 (2004).
38. Eichhorn, G. & Shin, Y. M. Interaction of metal ion with polynucleotides and related compounds. XII. The relative effect of various metal ions on DNA helicity. *J. Am. Chem. Soc.* **90**, 7323–7328 (1968).
39. Hud, N. V. (ed.) *Nucleic Acid–Metal Ion Interaction* (RSC Publishing, 2009).
40. Collins, P., Dobson, A. D. & Field, J. A. Reduction of the 2,2′-Azinobis (3-ethylbenzthiazoline-6-sulfonate) cation radical by physiological organic acids in the absence and presence of manganese. *Appl. Environ. Microbiol.* **64**, 2026–2031 (1998).
41. Ambrus, A. *et al.* Human telomeric sequence forms a hybrid-type intramolecular G-quadruplex structure with mixed parallel/antiparallel strands in potassium solution. *Nucleic Acids Res.* **34**, 2723–2735 (2006).
42. Masiero, S. *et al.* A non-empirical chromophoric interpretation of CD spectra of DNA G-quadruplex structures. *Org. Biomol. Chem.* **8**, 2683–2692 (2010).
43. Mori, K., Kodama, T., Baba, T. & Obika, S. Bridged nucleic acid conjugates at 6′-thiol: synthesis, hybridization properties and nuclease resistances. *Org. Biomol. Chem.* **9**, 5272–5279 (2011).
44. Nishioka, H., Liang, X., Kato, T. & Asanuma, H. A photo-fueled DNA nanodevices that contains two different photoswitches. *Angew. Chem. Int. Ed.* **51**, 1165–1168 (2012).
45. Rotaru, A. & Mokhir, A. Nucleic acid binders activated by light of selectable wavelength. *Angew. Chem. Int. Ed.* **46**, 6180–6183 (2007).
46. Arslan, P., Jyo, A. & Ihara, T. Reversible circularization of an anthracene-modified DNA conjugate through bimolecular triplex formation and its analytical application. *Org. Biomol. Chem.* **8**, 4843–4848 (2010).
47. Ogino, M. & Fujimoto, K. Photochemical synthesis of R-shaped DNA toward DNA recombination and processing in vitro. *Angew. Chem. Int. Ed.* **45**, 7223–7226 (2006).
48. Lim, R. K. V. & Lin, Q. Bioorthogonal chemistry: recent progress and future directions. *Chem. Commun.* **46**, 1589–1600 (2010).
49. Gramlich, P. M. E., Wirges, C. T., Manetto, A. & Carell, T. Postsynthetic DNA modification through the copper-catalyzed azide–alkyne cycloaddition reaction. *Angew. Chem. Int. Ed.* **47**, 8350–8358 (2008).
50. Mukae, M., Ihara, T., Tabara, M. & Jyo, A. Anthracene–DNA conjugates as building blocks of designed DNA structures constructed by photochemical reactions. *Org. Biomol. Chem.* **7**, 1349–1354 (2009).
51. Chen, X., Briggs, N., McLain, J. R. & Ellington, A. D. Stacking nonenzymatic circuits for high signal gain. *Proc. Natl Acad. Sci. USA* **110**, 5386–5391 (2013).
52. Ihara, T., Sueda, S., Inenaga, A., Fukuda, R. & Takagi, M. Synthetic DNA ligands conjugated with metal binding moiety. Regulation of the interaction with DNA by metal ions and the ligand effect on metal assisted DNA cleaving. *Supramol. Chem.* **8**, 93–111 (1997).
53. Ihara, T. *et al.* Metal ion-directed cooperative DNA binding of small molecules. *J. Inorg. Biochem.* **100**, 1744–1754 (2006).
54. Marky, L. A. & Breslauer, K. J. Calculating thermodynamic data for transition of any molecularity from equilibrium melting curve. *Biopolymers* **26**, 1601–1620 (1987).
55. Kitamura, Y. *et al.* Template-directed formation of luminescent lanthanide complexes: versatile tools for colorimetric identification of single nucleotide polymorphism. *J. Inorg. Biochem.* **102**, 1921–1931 (2008).
56. Cheng, X., Liu, X., Bing, T., Cao, Z. & Shangguan, D. General peroxidase activity of G-quadruplex–hemin complexes and its application in ligand screening. *Biochemistry* **48**, 7817–7823 (2009).
57. Li, C. *et al.* Backbone modification promotes peroxidase activity of G-quadruplex-based DNAzyme. *Chem. Commun.* **48**, 8347–8349 (2012).
58. Nakayama, S. & Sintim, H. O. Colorimetric split G-quadruplex probes for nucleic acid sensing: improving reconstituted DNAzyme’s catalytic efficiency via probe remodeling. *J. Am. Chem. Soc.* **131**, 10320–10333 (2009).
59. Kong, D.-M., Yang, W., Wu, J., Li, C. X. & Shen, H.-X. Structure-function study of peroxidase-like G-quadruplex-hemin complexes. *Analyst* **135**, 321–326 (2010).

Acknowledgements

We thank Ms M. Nishi for the assistance in the measurements of mass spectra for amide and conjugates identification. We also thank Professor H. Ihara and Professor M. Takafuji for their support in measuring CD spectra. This work was supported by the Grant-in-Aid for Scientific Research on Innovative Areas (Coordination Programming, Area 2107) (no. 24108734 to T.I.), Scientific Research (B) (no. 24350040 to T.I.) from the Ministry of Education, Culture, Sports, Science and Technology, Japan; and the Health Labor Sciences Research Grant (Research on Publicly Essential Drugs and Medical Devices) (to T.I.) from the Ministry of Health and Labor, Japan.

Author contribution

T.I. conceived the project, designed the systems and wrote the paper. H.O. synthesized the DNA conjugates and performed the DNAzyme activity measurements. C.S., T.F. and H.S. synthesized the amides and DNA conjugates and conducted the ultraviolet measurements. H.M. and Y.K. analysed and discussed the data.

Additional information

Supplementary Information accompanies this paper at <http://www.nature.com/naturecommunications>

Competing financial interests: The authors declare no competing financial interests.

Reprints and permission information is available online at <http://npg.nature.com/reprintsandpermissions/>

How to cite this article: Ihara T. *et al.* Metal ion-directed dynamic splicing of DNA through global conformational change by intramolecular complexation. *Nat. Commun.* 6:6640 doi: 10.1038/ncomms7640 (2015).