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A supramolecular DNA self-assembly based on β-cyclodextrin–adamantane complexation as a bioorthogonal sticky end motif†

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We propose linear end-to-end assemblies of short DNA duplexes based on β-cyclodextrin–adamantane complexation. The assembled duplexes exhibited increased T_m values compared with those of the corresponding natural hybrids. Competition experiments with external guest molecules showed a substantial decrease in T_m of the terminal modified duplexes, suggesting the viability of inter-duplex complexation.

DNA is a unique nanoconstruction tool for rational molecular architectures because of its ease of synthesis and base-pairing loyalty with sequence programmability. Attachment of artificial functional components to natural DNA has led to evolution of fields of supramolecular chemistry, DNA nanotechnology, and chemical biology.[1] An attractive approach to set up functionalized DNA is to conjugate natural DNA scaffolds with host–guest (H–G) chemistry. We have reported DNA duplex-based, versatile fluorescent sensors consisting of three functional regions, *i.e.*, host molecules such as crown ethers and cyclodextrins (CDs), DNA, and fluorescent pyrenes.[2] Presence of the corresponding guest molecules induced emission switching from a monomer to an excimer of the pyrenes due to the DNA hybridization promoted by H–G association.

Among the various DNA structures, one-dimensional long structures assembling with short oligomers are fundamental structural motifs of DNA nanoarchitecture. Traditional approaches rely on Watson–Crick base pairing using thermodynamic assembly of "sticky ends".[3] Kinetically controlled self-assembly of natural oligomers was also reported to build one-dimensional linear DNA.[4] End-to-end double helix assembly based on hydrophobic interaction of terminal base pairs was observed at high concentrations in single crystals and in liquid crystals.[5] In dilute solution, hydrophobic self-assembly of a perylenediimide-linked DNA dumbbell was recently reported, in which linear end-to-end assemblies of 10–30 dumbbell monomers as well as bundles of the linear polymers formed.[6] However, well-defined H–G complexation has not been employed for the construction of linear DNA supramolecular polymers. If such H–G complexation could be utilized as a sticky end in DNA termini, this bioorthogonal technique may offer an additional strategy for constructing DNA nanoarchitecture. Because we can choose a H–G pair from various types of H–G combinations, we may control the association and the external-stimulus dissociation of supramolecular DNA polymers. Here we report an end-to-end type supramolecular DNA self-assembly based on β-cyclodextrin–adamantane (β-CD–Ad) association attached to both the ends of DNA duplexes.

In our previous sensors, CDs as the guest-binding moiety were attached to the termini of DNA duplexes, and the bis-Ads guest molecule was externally added to the DNA solution. We expected that direct introduction of CD and Ad groups into both the 5′-ends of ds-DNA would induce end-to-end assembly of the short ds-DNA through inter-duplex β-CD–Ad complexation, affording a linear supramolecular structure (Fig. 1 and Fig. S1, ESI†). Thus, we designed 5′-β-CD- and 5′-Ad-modified complementary oligodeoxynucleotides (ODNs). According to our previous procedure,[2] the ODNs were synthesized by Huisgen reaction[7] between the alkynylnucleoside residue[8] reported by

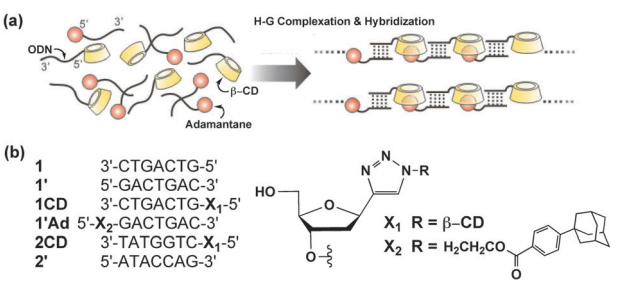

Fig. 1 (a) Schematic illustration of an end-to-end self-association of DNA hybrids based on β-cyclodextrin–adamantane (β-CD–Ad) complexation. (b) Sequences of ODNs **1**, **1′**, **1CD**, **1′Ad**, **2CD**, and **2′**. X represents nucleoside residues of Huisgen products between alkynylnucleoside and azido derivatives of β-CD **3** or Ad **7**.

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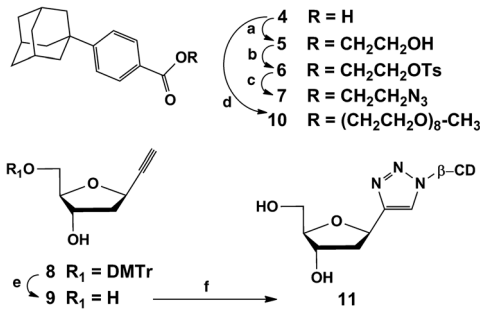

Scheme 1 (a) $HOCH_2CH_2OH$, EDC, DMAP, CH_2Cl_2; 93%, (b) *p*-TsCl, DMAP, pyridine; 90%, (c) NaN_3, DMF; 98%, (d) $HO(CH_2CH_2O)_8CH_3$, EDC, DMAP, CH_2Cl_2; 70%, (e) CCl_3CO_2H, CH_2Cl_2; 88%, (f) **3**, CuCl, H_2O; 89%.

us and azide derivatives of β-CD **3** (ref. 9) or Ad **7**. The crude ODNs were purified by reverse-phase HPLC and characterized by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass measurements (see ESI†). For an azide derivative of Ad, **7** was newly synthesized in good overall yield from 4-adamantyl benzoic acid **4** (ref. 10) *via* esterification, tosylation, and azidation steps (Scheme 1).

First, we evaluated thermal stability of the duplexes for **1CD–1′Ad** and the corresponding natural **1–1′**. Based on the first derivative profiles of the UV-melting curves of the two duplexes (Fig. S2, ESI†), melting temperatures (T_m) of 45 °C for **1CD–1′Ad** and 27 °C for **1–1′** were obtained (see also Table 1, entries 1 and 10). The drastic increase in the thermal stability (ΔT_m = 18 °C) suggested that β-CD and Ad moieties in **1CD–1′Ad** significantly take part, probably form H–G complexes, in the higher-order structure formation between **1CD** and **1′Ad**.

In order to confirm the definite complexation between the β-CD and Ad moieties in the measurement concentration range of 10^{-5} in water, we prepared two model compounds **10** and **11** (Scheme 1). The self-association of **10** was judged to be negligible at $\leq$60 μM by a UV dilution experiment, so that the following titration assays were carried out below that concentration. The addition of **11** into an aqueous solution of **10** (40 μM) caused significant spectral change in UV measurements; the absorbance at 245 nm gradually decreased, while that at 210 nm increased (Fig. S3a, ESI†). This alteration was accompanied by an isosbestic point at 240 nm, which indicated that only two absorptive species, the monomeric and the associated states of **10**, exist in the solution. In the curve-fitting analysis, a well-fitted theoretical curve could be drawn on the basis of the assumption of 1 : 1 complexation between **10** and **11**, and the dissociation constant was obtained to be $K_d = 1.5 \times 10^{-7}$ M (Fig. S3b, ESI†). In addition, isothermal titration calorimetry (ITC) measurements were carried out for the same combination of **10** and **11**, which afforded a K_d of 8.7×10^{-7} M ($\Delta G_{293} = -8.2$ kcal mol^{-1}, $\Delta H = -9.7$ kcal mol^{-1}, and $\Delta S = -5.1$ cal K^{-1} mol^{-1}) (Fig. S3c, ESI†). These two dissociation constants are comparable to the reported K_d values for complexes between β-CD and phenyl-substituted Ad derivatives in water.[11] Considering from the $\sim 10^{-7}$ M order of the K_d values, we concluded that almost all of the β-CD and Ad moieties would form the inter-strand complex between **1CD** and **1′Ad** in the UV-melting experiments in the concentration range of 10^{-5} M.

Next, we attempted competition experiments for the duplex formation on **1CD–1′Ad**, **1–1′Ad**, **1CD–1′**, and **1–1′** with 1-adamantanecarboxylic acid or β-CD as a competitor (Table 1). Duplexes lacking in CD (**1–1′Ad**) or Ad (**1CD–1′**) as well as natural **1–1′** showed no obvious T_m changes upon addition of the competitors (entries 4–12). On the other hand, substantial T_m decreases were observed for **1CD–1′Ad** by the addition of 1 mM 1-adamantanecarboxylic acid or β-CD (entries 2 and 3), verifying the inter-duplex β-CD–Ad complexation for **1CD–1′Ad**.

We prepared two natural ODNs of 3′-CTGACTG*TTTGGGG*-5′ and 5′-*AAACCCC*GACTGAC-3′ as the corresponding natural sticky-end polymerizing component with a 7 mer core duplex. In the ODNs, italicized and underlined residues are complementary respectively, and the underlined combination has the same sequence as the core 7 mer of **1–1′**. A thermal denaturation experiment for the duplex of 3′-CTGACTG*TTTGGGG*-5′/5′-*AAACCCC*GACTGAC-3′ ([ssDNA] = 8 μM, 20 mM Tris-HCl (pH 8.0), 100 mM NaCl, 1 °C min^{-1}, path length = 10 mm) showed the increased thermal stability (ΔT_m = 5.5 °C) compared with that of the core 7 mer **1–1′**. The adjacent core 7 mer-duplexes in the natural sticky-end polymer would interact with each other by end-to-end stacking of the terminal base pairs. This may be one of the reasons for the observed stabilization as seen in the increased thermal stability of dangling ends. At the present stage, it is difficult to clearly envisage the molecular basis for the reason why **1CD–1′Ad** showed further thermal stability beyond the corresponding natural sticky-end polymer. We speculate that the CD–Ad complexes interact with the terminal base pairs of the core duplexes in good orientation avoiding destabilization based on steric hindrance of the complexes.

To investigate the effect of the lengths of DNA helices, we further prepared several β-CD- and Ad-modified ODNs (**12CD**, **12′Ad**, **13CD**, **13′Ad**, **14CD**, **14′Ad**, **15CD**, **15′Ad**, **16CD**, and **16′Ad**) with different 5–14 mer lengths (see ESI† and Table S1). All of the modified duplexes **12CD–12′Ad** (14 mer), **13CD–13′Ad** (10 mer), **14CD–14′Ad** (8 mer), **1CD–1′Ad** (7 mer), **15CD–15′Ad** (6 mer), and **16CD–16′Ad** (5 mer) exhibited increased T_m values compared with those of the corresponding natural hybrids **12–12′**,

Table 1 Competition experiments on $AdCO_2H$ or β-CD for duplexes **1CD–1′Ad**, **1–1′Ad**, **1CD–1′**, and **1–1′**

Entry	Duplex	Additive	T_m (°C)
1	**1CD–1′Ad**	—	45
2	**1CD–1′Ad**	1 mM $AdCO_2H$	38
3	**1CD–1′Ad**	1 mM β-CD	33.5
4	**1–1′Ad**	—	32
5	**1–1′Ad**	1 mM $AdCO_2H$	32
6	**1–1′Ad**	1 mM β-CD	30.5
7	**1CD–1′**	—	30.5
8	**1CD–1′**	1 mM $AdCO_2H$	31
9	**1CD–1′**	1 mM β-CD	30
10	**1–1′**	—	27
11	**1–1′**	1 mM $AdCO_2H$	27
12	**1–1′**	1 mM β-CD	26.5

[ssDNA] = 10 μM, 20 mM Tris-HCl (pH 8.0), 100 mM NaCl, 1 °C min^{-1}, path length = 10 mm.

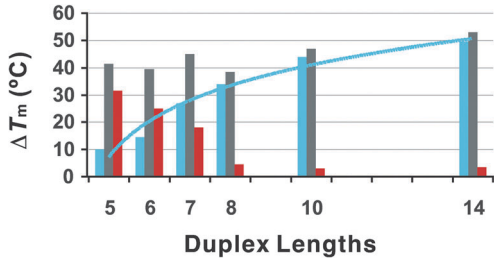

Fig. 2 Duplex lengths *versus* T_m values for natural (blue) and modified (gray) DNA. The red bars indicate the ΔT_m values. [ssDNA] = 10 μM, 20 mM Tris-HCl (pH 8.0), 100 mM NaCl, 1 °C min^{-1}, path length = 10 mm.

13–13′, **14–14′**, **1–1′**, **15–15′**, and **16–16′**, respectively (Fig. 2). Despite the difference in the component DNA lengths, β-CD- and Ad-modified duplexes displayed roughly similar melting temperatures of around 40–50 °C, suggesting that the self-assembled supramolecular duplexes possess almost the same thermal stability. Because natural DNA inherently has an asymptotic T_m value as the length increases, it is not surprising that the self-assembled supramolecular duplexes show almost the same thermal stability. In other words, both the modified shorter and longer duplexes might form similar lengths of the supramolecular self-assemblies, which are near to sufficiently long natural duplexes, at least of *ca.* 14 mer length. Therefore, the shorter the component duplex, the larger ΔT_m was observed because the short natural hybrids have substantially low thermal stability for the duplex formation.

In conclusion, we have synthesized 5′-β-CD- and 5′-Ad-modified complementary ODNs. The duplexes exhibited increased T_m values compared with those of the corresponding natural hybrids. The H–G association was confirmed by competition experiments for the modified duplexes. Moreover, two types of titration experiments based on model compounds also exhibited the definite β-CD–Ad complexation in water. Taken together, we suggest that a supramolecular end-to-end self-assembly of short DNA duplexes would be induced by H–G complexation even in dilute aqueous solution. More detailed analysis of the higher order structure and additional advancements of this bioorthogonal technique are now in progress for constructing DNA nanoarchitecture in which an additional strategy by H–G chemistry is utilized.

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