Synthesis of covalently linked parallel and antiparallel DNA duplexes containing the metal-mediated base pairs T–Hg(II)–T and C–Ag(I)–C†

Takashi Ono, Kyohei Yoshida, Yuko Saotome, Rei Sakabe, Itaru Okamoto and Akira Ono*

Received 23rd June 2010, Accepted 4th November 2010
DOI: 10.1039/c0cc02028a

DNA duplexes fixed in anti-parallel and parallel orientations by introducing covalent linkages have been synthesized and metal ions, Hg(II) and Ag(I), were incorporated into pyrimidine–pyrimidine base pairs.

We report a method for the synthesis of highly stabilized, short DNA duplexes in which the DNA strands are held in an antiparallel or parallel orientation by inter-strand covalent linkages. Previously developed methods for the production of covalently linked nucleotides have led to investigations of the potential of linked nucleotides to act as decoy DNAs[1] and antisense and anti-gene oligonucleotides.[2] As structural mimics of the damaged sites induced by bi-functional mutagenic chemicals, inter-strand cross-linked duplexes have also been used to investigate the process of DNA damage repair.[3]

One approach for preparing covalently linked DNA duplexes consists of several steps: first, oligonucleotides containing artificial reactive residues are synthesized; the synthesized oligonucleotides are then hybridized to form a duplex, and finally the reactive residues in close proximity are chemically conjugated. This approach has been used to prepare duplexes with various covalent linkages, including disulfide, psoralen, malondialdehyde, and alkyl groups.[4] An alternative approach is the direct synthesis of covalently linked oligonucleotides on an automatic DNA/RNA synthesizer using amidite units of a nucleoside dimer linking the base residues of two nucleosides.[5] In pioneering studies carried out by the Hopkins,[5a] Miller,[5d–i] and Kishi[5b] groups, amidite units of linked nucleosides were used to synthesize duplexes with covalent linkages in the middle of the duplexes using sophisticated protecting groups and the multi-step synthetic procedures. In many of these pioneering studies, linkages were introduced inside the duplexes,[6] and none have reported the preparation of covalently linked duplexes with parallel strands.[7] In this report, we describe efficient methods for the synthesis of duplexes that are fixed in an antiparallel or parallel orientation by covalent linkages placed at the duplex ends. We also describe the utility of this method through the application of linked duplexes, allowing the demonstration of metal ion-mediated base pair formation[8] in antiparallel and parallel duplexes.

When mixed, homothymidylic acid (Tn) and homodeoxyadenylic acid (dAn) intrinsically form antiparallel and parallel duplexes. In antiparallel-strand (*aps*) duplexes, adenine (A) residues and thymine (T) residues form A–T pairs of Watson–Crick (W–C) geometry. On the other hand, in parallel-strand (*ps*) duplexes, A–T pairs are formed in reverse W–C geometry (Fig. 1). Since *aps* duplexes are more stable than *ps* duplexes, dAn and Tn form *aps* duplexes under physiological conditions. However, *ps* duplexes can be formed with meaningful stability if the strand orientation is fixed in parallel using a hairpin structure.[7] The previously reported hairpin *ps*-duplex contained a 5′–5′ phosphodiester linkage at a hairpin–stem junction, and the effects of this unique structure on the stability of the duplex were not examined. Here, we have developed a more sophisticated method for synthesizing *aps* and *ps* duplexes in which the strand orientations are fixed by attaching the ethylene-linked pair T–(Et)–T at the duplex ends. The introduction of T–(Et)–T inside an *aps* duplex has been reported to efficiently stabilize it.[5g] We expected that rotation of the thymine residues around the ethylene linker would stabilize the reverse W–C geometry, efficiently stabilizing *ps* duplexes.

Structures of novel phosphoramidite units of thymidine dimers are shown in Fig. 2. In these phosphoramidite units, the 3N positions of two thymidines are linked with ethylene. The phosphoramidites **1** and **2**, which are used for the preparation of *aps* and *ps* duplexes, respectively, have similar structures; they differ only in the positions of the protecting groups on the sugar residues of the nucleosides shown at left.

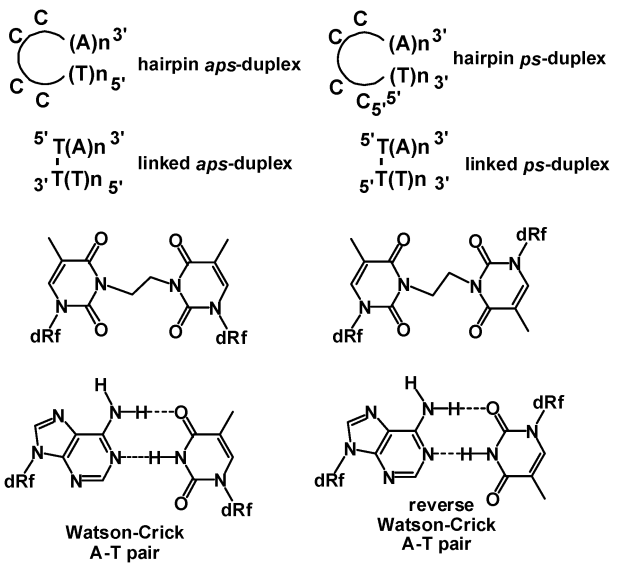

Fig. 1 Base-pairing geometry of A–T pairs and covalently linked T–(Et)–T pairs in *aps* and *ps* duplexes.

Department of Material & Life Chemistry, Faculty of Engineering, Kanagawa University, 3-27-1 Rokkakubashi, Kanagawa-ku, Yokohama, Kanagawa 221-8686, Japan.
E-mail: akiraono@kanagawa-u.ac.jp; Fax: +81 454917915; Tel: +81 454815661

† Electronic supplementary information (ESI) available: Synthesis of amidite units of thymidine dimers, oligonucleotides, thermal denaturation studies. See DOI: 10.1039/c0cc02028a

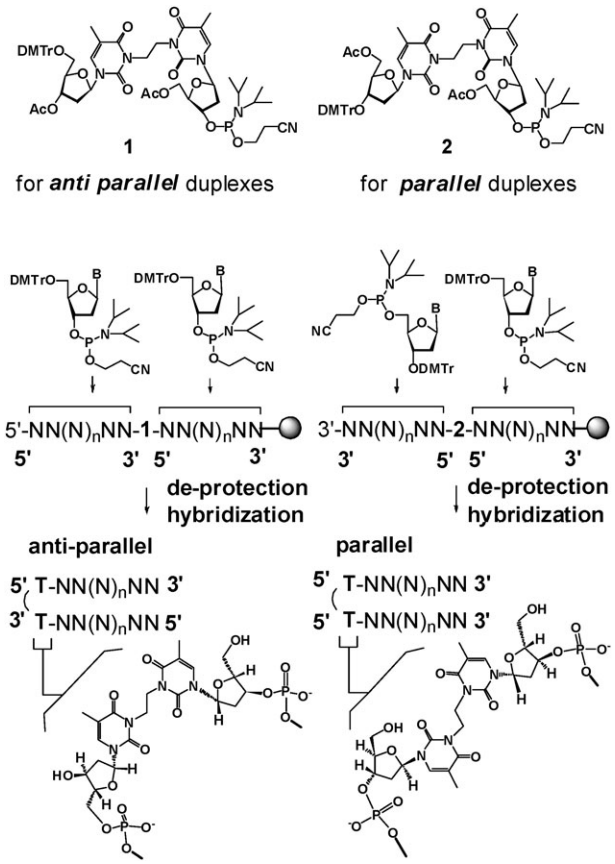

Fig. 2 Upper: structures of the linked thymidine dimer phosphoramidite unit **1** (for synthesizing *aps* duplex) and **2** (for *ps* duplex). Lower: schematic representations of the synthetic strategies for *aps* (left) and *ps* (right) linked duplexes.

Covalently linked oligodeoxyribonucleotides (ODNs) were synthesized on a DNA/RNA synthesizer using a general protocol recommended by the manufacturer, with slight modifications. Schemes for the synthesis of covalently linked duplexes in the antiparallel and the parallel orientations are shown schematically in Fig. 2. The procedure for synthesizing linked *aps* duplexes is as follows: starting with 5′-*O*-DMTr-nucleoside attached to the solid support, the ODN is elongated using commercially available nucleoside-3′-*O*-phosphoramidite units; then, phosphoramidite **1** is attached, and a second strand is elongated using nucleoside-3′-*O*-phosphoramidite units. For synthesizing linked *ps* duplexes, after **2** is attached, 5′-*O*-phosphoramidite units are used in the final elongation step, in which a second strand is elongated from the 5′-end to the 3′-end. The fully protected ODNs are deprotected and purified by the same procedures as those used for unmodified ODNs.

The thermal stabilities of the linked duplexes were compared to those of hairpin duplexes. The thermally induced transition profiles of the linked and hairpin duplexes are shown in Fig. 3. The linked *aps* and *ps* duplexes were of similar stability, and the T_ms of the linked duplexes (46 °C for *aps* and 47 °C for *ps*) were higher than those of the hairpin duplexes (29 °C for *aps* and 24 °C for *ps*). Thus, the covalent linkage attached at the duplex end efficiently stabilized both the *aps* and *ps* duplexes. The hairpin *aps* duplex displayed

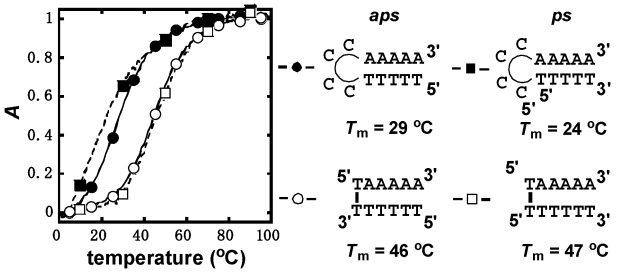

Fig. 3 Thermally induced transition profiles of ODNs (5 μM) in 10 mM sodium cacodylate buffer (pH = 7.0) containing 100 mM NaCl.

a T_m higher than that of the hairpin *ps* duplex as previously demonstrated.[7a] Consequently, as a good fit into the *ps* duplex structure was observed, we speculate that the T–(Et)–T linkage efficiently stabilizes the *ps* duplex.

The linked duplexes were applied to the determination of possible geometries of metal-mediated base pairs.[8] T–T and C–C mismatches in DNA duplexes selectively capture Hg(II) ions and Ag(I) ions, respectively, forming metal-mediated T–Hg(II)–T and C–Ag(I)–C base pairs (Fig. 4). Using ^{15}N-NMR spectroscopy, we verified the formation of the 3*N*-Hg(II) bonding in the T–Hg(II)–T structure. As shown in Fig. 5 right, the metal ion mediated base pairs can be formed in reverse W–C geometry, probably in *ps* duplexes.

The thermally induced transition profiles of the linked duplexes containing pyrimidine–pyrimidine (T–T and C–C) mismatches in the absence and presence of metal ions are shown in Fig. 5. As previously reported, *aps* duplexes were stabilized by the presence of metal ions. The *ps*-duplexes were also stabilized by the presence of metal ions, indicating the formation of T–Hg(II)–T and C–Ag(I)–C in reverse W–C geometry.[9] The formation of the metal ion mediated base pairs was also detected by mass spectrometry (ESI†).

In conclusion, we have developed a method for synthesizing covalently linked, stable *aps* and *ps* duplexes. These duplexes are anticipated as being useful for developing biologically active nucleic acids, and for structural studies. We demonstrated the utility of the linked duplexes by showing formation of the metal ion mediated pyrimidine base pairs in reverse W–C geometry in duplexes for the first time.

This study was partly supported by a Grant-in-Aid for Scientific Research (B) (21350105) and the Science Frontier

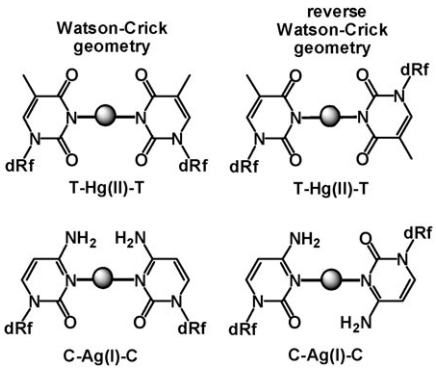

Fig. 4 Proposed structures of the metal ion-mediated base pairs T–Hg(II)–T and C–Ag(I)–C in W–C (left) and reverse W–C (right) geometry.

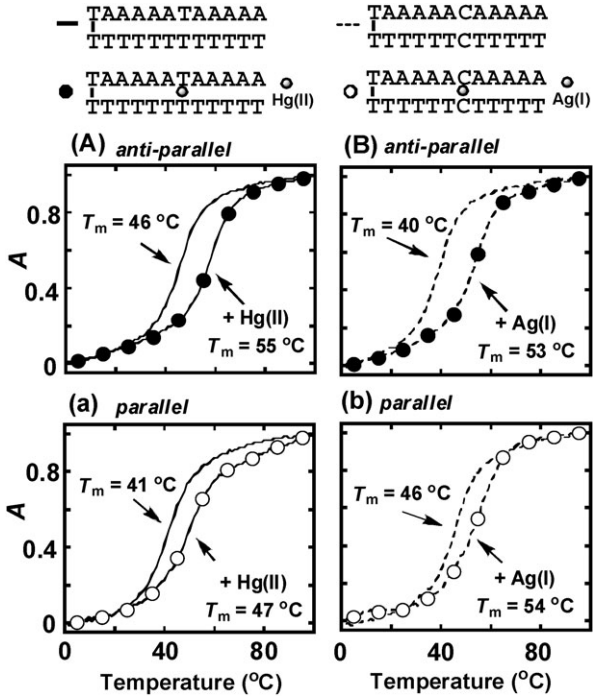

Fig. 5 Thermally induced transition profiles of linked duplexes containing T–T and C–C mismatches. ODNs (2 μM) and metal ions (2.4 μM) were dissolved in 10 mM MOPS buffer (pH = 7.1) containing 100 mM NaCl (see ESI†).

"Construction and control of chemical space purposing development of new functionalized molecules and materials" (2006–2011) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan.

Notes and references

1 (*a*) A. Murakami, Y. Yamamoto, M. Namba, R. Iwase and T. Yamaoka, *Bioorg. Chem.*, 2001, **29**, 223–233; (*b*) M. Nakane, S. Ichikawa and A. J. Matsuda, *J. Org. Chem.*, 2008, **73**, 358–366.

2 (*a*) A. Sasaki and F. Nagatsugi, *Curr. Opin. Chem. Biol.*, 2006, **10**, 615–621; (*b*) Z. Qiu, L. Lu, X. Jian and C. He, *J. Am. Chem. Soc.*, 2008, **130**, 14398–14399; (*c*) M. A. Campbell and P. S. Miller, *JBIC, J. Biol. Inorg. Chem.*, 2009, **14**, 873–881.

3 (*a*) J. T. Sczepanski, A. C. Jacobs, B. Van Houten and M. M. Greenberg, *Biochemistry*, 2009, **48**, 7565–7567; (*b*) M. B. Smeaton, E. M. Hlavin, A. M. Noronha, S. P. Murphy, C. J. Wilds and P. S. Miller, *Chem. Res. Toxicol.*, 2009, **22**, 1285–1297; (*c*) Q. Fang, A. M. Noronha, S. P. Murphy, C. J. Wilds, J. L. Tubbs, J. A. Tainer, G. Chowdhury, F. P. Guengerich and A. E. Pegg, *Biochemistry*, 2008, **47**, 10892–10903; (*d*) I. G. Minko, M. B. Harbut, I. D. Kozekov, P. M. Jakobs, S. B. Olson, R. E. Moses, T. M. Harris, C. J. Rizzo and R. S. Lloyd, *J. Biol. Chem.*, 2008, **283**, 17075–17082.

4 (*a*) A. E. Ferentz, T. E. Keating and G. L. Verdine, *J. Am. Chem. Soc.*, 1993, **115**, 9006–9014; (*b*) D. A. Erlanson, L. Chen and G. L. Verdine, *J. Am. Chem. Soc.*, 1993, **115**, 12583–12584; (*c*) W. R. Kobertz and J. M. Essigmann, *J. Am. Chem. Soc.*, 1997, **119**, 5960–5961; (*d*) X. Peng, I. S. Hong, H. Li, M. M. Seidman and M. M. Greenberg, *J. Am. Chem. Soc.*, 2008, **130**, 10299–10306; (*e*) H. Ding, A. Majumdar, J. R. Tolman and M. M. Greenberg, *J. Am. Chem. Soc.*, 2008, **130**, 17981–17987; (*f*) J. T. Sczepanski, A. C. Jacobs, A. Majumdar and M. M. Greenberg, *J. Am. Chem. Soc.*, 2009, **131**, 11132–11139; (*g*) P. A. Dooley, D. Tsarouhtsis, G. A. Korbel, L. V. Nechev, J. Shearer, I. S. Zegar, C. M. Harris, M. P. Stone and T. M. Harris, *J. Am. Chem. Soc.*, 2001, **123**, 1730–1739; (*h*) P. A. Dooley, M. Zhang, G. A. Korbel, L. V. Nechev, C. M. Harris, M. P. Stone and T. M. Harris, *J. Am. Chem. Soc.*, 2003, **125**, 62–72; (*i*) M. P. Stone, Y. Cho, H. Huang, H. Y. Kim, I. D. Kozekov, A. Kozekova, H. Wang, I. G. Minko, R. S. Lloyd, T. M. Harris and C. J. Rizzo, *Acc. Chem. Res.*, 2008, **41**, 793–804; (*j*) H. Huang, H. Y. Kim, I. D. Kozekov, Y. J. Cho, H. Wang, A. Kozekova, T. M. Harris, C. J. Rizzo and M. P. Stone, *J. Am. Chem. Soc.*, 2009, **131**, 8416–8424; (*k*) K. Stevens and A. Madder, *Nucleic Acids Res.*, 2009, **37**, 1555–1565; (*l*) T. Angelov, A. Guainazzi and O. D. Schärer, *Org. Lett.*, 2009, **11**, 661–664; (*m*) Y. Yoshimura and K. Fujimoto, *Org. Lett.*, 2008, **10**, 3227–3230; (*n*) A. Kobori, K. Takaya, M. Higuchi, A. Yamayoshi and A. Murakami, *Chem. Lett.*, 2009, 272–273.

5 (*a*) E. A. Harwood, S. T. Sigurdsson, N. B. F. Edfeldt, B. R. Reid and P. B. Hopkins, *J. Am. Chem. Soc.*, 1999, **121**, 5081–5082; (*b*) H. Y. Li, Y. L. Qiu, E. Moyroud and Y. Kishi, *Angew. Chem., Int. Ed.*, 2001, **40**, 1471–1475; (*c*) H. Y. Li, Y. L. Qiu and Y. Kishi, *ChemBioChem*, 2001, **2**, 371–374; (*d*) D. M. Noll, A. M. Noronha and P. S. Miller, *J. Am. Chem. Soc.*, 2001, **123**, 3405–3411; (*e*) A. M. Noronha, D. M. Noll, C. J. Wilds and P. S. Miller, *Biochemistry*, 2002, **41**, 760–771; (*f*) A. M. Noronha, C. J. Wilds and P. S. Miller, *Biochemistry*, 2002, **41**, 8605–8612; (*g*) C. J. Wilds, A. M. Noronha, S. Robidoux and P. S. Miller, *J. Am. Chem. Soc.*, 2004, **126**, 9257–9265; (*h*) C. J. Wilds, E. Palus and A. Noronha, *Can. J. Chem.*, 2007, **85**, 249–256; (*i*) C. J. Wilds, F. Xu and A. M. Noronha, *Chem. Res. Toxicol.*, 2008, **21**, 686–695.

6 As described in ref. 5*b* and 5*c*, Li and coworkers synthesized a duplex with a covalently linked base pair at the duplex end, and this link highly stabilized the duplex.

7 (*a*) J. H. van de Sande, N. B. Ramsing, M. W. Germann, W. Elhorst, B. W. Kalisch, E. V. Kitzing, R. T. Pon, R. C. Clegg and T. M. Jovin, *Science*, 1988, **241**, 551–557; (*b*) I. Förtsch, H. Fritzsche, E. Birch-Hirschfeld, E. Evertsz, R. Klement, T. M. Jovin and C. Zimmer, *Biopolymers*, 1996, **38**, 209–220.

8 (*a*) Y. Miyake, H. Togashi, M. Tashiro, H. Yamaguchi, S. Oda, M. Kudo, Y. Tanaka, Y. Kondo, R. Sawa, T. Fujimoto, T. Machinami and A. Ono, *J. Am. Chem. Soc.*, 2006, **128**, 2172–2173; (*b*) Y. Tanaka, S. Oda, H. Yamaguchi, Y. Kondo, C. Kojima and A. Ono, *J. Am. Chem. Soc.*, 2007, **129**, 244–245; (*c*) A. Ono and H. Togashi, *Angew. Chem., Int. Ed.*, 2004, **43**, 4300–4302; (*d*) A. Ono, S. Cao, H. Togashi, M. Tashiro, T. Fujimoto, T. Machinami, S. Oda, Y. Miyake, I. Okamoto and Y. Tanaka, *Chem. Commun.*, 2008, 4825–4827; (*e*) G. H. Clever, C. Kaul and T. Carell, *Angew. Chem., Int. Ed.*, 2007, **46**, 6226–6236; (*f*) K. Tanaka, G. H. Clever, Y. Takezawa, Y. Yamada, C. Kaul, M. Shionoya and T. Carell, *Nat. Nanotechnol.*, 2006, **1**, 190–194.

9 Some studies have reported metal ion binding to Hoogsteen type base pairs in parallel duplex and triplex strands. (*a*) D. A. Megger and J. Müller, *Nucleosides, Nucleotides Nucleic Acids*, 2010, **29**, 27–38; (*b*) T. Ihara, T. Ishii, N. Araki, A. W. Wilson and A. Jyo, *J. Am. Chem. Soc.*, 2009, **131**, 3826–3827.

