Thermodynamic properties of branched DNA complexes with full-matched and mismatched DNA strands†

Masayuki Endo* and Tetsuro Majima*

Received (in Cambridge, UK) 30th January 2006, Accepted 3rd April 2006
First published as an Advance Article on the web 19th April 2006
DOI: 10.1039/b601372d

Complexes consisting of a branched DNA with full-matched and mismatched DNA strands were prepared, and the cross-linking of the DNA strands and their diastereochemistry affected the stability of the complexes and the thermodynamics of the complex formation.

Conformational switching between the structural variants of DNA, which is induced by the specific DNA molecules and co-factors, has attracted considerable attention because these systems can be used for the construction of mechanical devices.[1–7] In addition, an effective use of DNA structural variants is a molecular beacon system, in which a metastable hairpin DNA can be linearized by a target DNA strand which is complementary to the loop region of the beacon.[8,9] In this case, the analyte DNA having a mismatched base cannot hybridize to the beacon, resulting in discrimination between the full-matched and mismatched sequences using the FRET-based detection method.[8,9] For further extension of the structural variations for the detection of the mismatched bases, a novel DNA "host", which can discriminate base-mismatches, should be developed.

In our previous work, we prepared a cross-linked DNA (XL-DNA), in which two single DNA strands were connected by a disulfide linkage and bismaleimide linker, and controlled the arrangements and orientations of the multiple duplexes using the XL-DNA molecules.[10–13] In these studies, we found that the DNA complex containing one XL-DNA with a DNA strand complementary to one of the two strands of XL-DNA showed poor stability, while the complexes containing two XL-DNA molecules and complementary strand formed stable complexes.[10,11] We also noticed that one XL-DNA with a DNA strand, which is complementary to both strands of the XL-DNA, formed stable complexes.[10] For utilizing this unique phenomenon, we prepared an XL-DNA and counterpart DNA strands having mismatched bases to investigate the properties of the complex formation and dissociation.

In the present study, we examined the thermodynamic behavior of the DNA complexes containing a branched DNA connected by an interstrand cross-linker (Fig. 1). Two 10mer DNA strands were connected at the central phosphorus atom by a cross-linker having a disulfide linkage (Fig. 1a). We also used the native 20mer DNA having the same sequence as that of the XL-DNA (Fig. 1b). First, we employed the complementary strand **3**-TA for hybridization to the XL-DNA and native 20mer strand (Fig. 1c). In addition, single-mismatched bases were introduced to the counterpart strands (**3**-TX), and multiple mismatched thymidines (T) were also inserted in the indicated positions (Fig. 1c). The XL-DNA was prepared according to a previously reported procedure.[10–14] Because a linker is introduced to a phosphorus atom, two adjacent diastereomer peaks are generated, and the faster and slower eluted diastereomers on a reversed-phase HPLC are denoted as diastereomers A and B, respectively (Fig. S1, Supplementary Material†), and their configurations were estimated as S_p- and R_p-one, respectively.[14,15] The production of XL-DNAs **1A** and **1B** was confirmed by MALDI-TOF mass spectroscopy (Fig. S2†).[16]

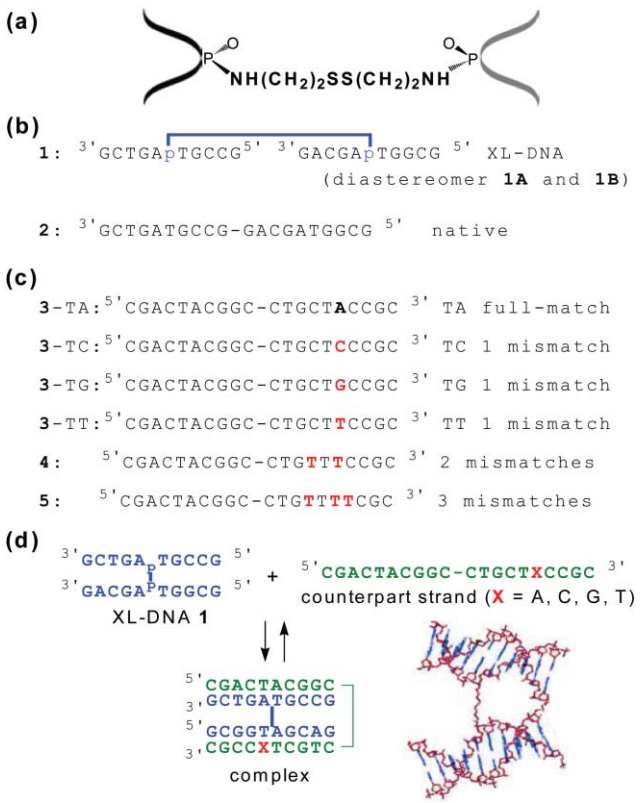

Fig. 1 Branched DNA employed in the experiment and sequences of DNA strands. (a) Structure of a branched disulfide cross-linked DNA (XL-DNA). (b) Sequences of XL-DNAs **1** and native DNA **2**. Bold bar represents a cross-linker. (c) Sequences of the counterpart strands **3**-TX, **4**, and **5** for XL-DNA and native DNA. Mismatched bases were introduced to the indicated positions. (d) Complex formation of XL-DNA with its counterpart strand and the model of the DNA complex.

The Institute of Scientific and Industrial Research, Osaka University, 8-1 Mihogaoka, Ibaraki, Osaka, 567-0047, Japan.
E-mail: endo@sanken.osaka-u.ac.jp; majima@sanken.osaka-u.ac.jp
† Electronic supplementary information (ESI) available: Synthesis of XL-DNAs, HPLC profiles, MALDI-TOF MS data, and melting profiles. See DOI: 10.1039/b601372d

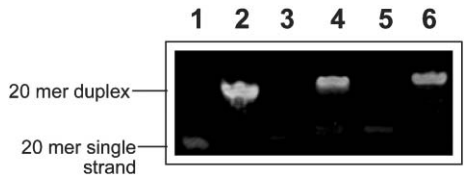

Fig. 2 Non-denaturing PAGE analysis of the complexes containing the XL-DNA and complementary strand. Lane 1, 20mer single strand (**3**-TA); lane 2, 20mer duplex (**2/3**-TA); lane 3, **1A**; lane 4, **1A** + **3**-TA; lane 5, **1B**; lane 6, **1B** + **3**-TA.

The formation of the complexes containing XL-DNA and its complementary strand was examined using non-denaturing polyacrylamide gel electrophoresis (PAGE) (Fig. 2).[17] In both cases of **1A** (lane 4) and **1B** (lane 6) with the complementary strand **3**-TA, complex formations were observed, and the complexes migrated slightly slower than those of the native 20mer **2/3**-TA duplex (lane 2). The XL-DNA forms a non-canonical complex structure as compared to the usual 20mer duplex and is less negatively charged (16 negative charges) than the 20mer strand (19 negative charges), which may affect the slower mobility in the non-denaturing polyacrylamide gel.

To examine the thermodynamic properties of the complexes containing XL-DNA, the melting temperatures of various concentrations of the complexes were measued for the analysis using van't Hoff plots.[18–21] The thermodynamic parameters of these complexes are summarized in Table 1. For the unmodified 20mer duplexes with full-matched and single mismatched sequences, the T_m values decreased by 3.7–6.8 °C as compared to those of the native duplex. The differences in the enthalpy and entropy changes between the full-match and mismatch were 17–21 kJ mol^{-1} and 0.01–0.04 kJ mol^{-1} K^{-1}, respectively, and these values corresponded to the single-base mismatches of the normal duplexes under the expermental conditions.

For the complexes of XL-DNA with the full-matched **3**-TA strand, the T_m values of **1A** and **1B** with **3**-TA significantly decreased by 22.9 and 18.5 °C compared with the native 20mer **2/3**-TA duplex, respectively. The thermodynamic parameters of the complexes containing **1A**, **1B**, and **2** were compared. The XL-DNA complexes showed a large difference in the entropy change as compared to that of the native duplexes, indicating the difference in the flexibility of the XL-DNA molecules. Also the difference in the entropy change between the complexes of **1A** and **1B** corresponds to the strain caused by the different configurations of the phosphoramidates in the phosphate–sugar backbones.

Table 2 Melting temperatures of the complexes of XL-DNA with the counterpart strands containing multiple mismatched bases[18]

DNA	**4** 2 mismatches	ΔT_m[a]/°C	**5** 3 mismatches	ΔT_m[a]/°C
1A	37.4	−20.3	34.0	−18.7
1B	42.6	−15.1	37.2	−15.5
2	57.7	—	52.7	—

[a] The ΔT_m values are the difference between the complexes containing XL-DNA with the **4** or **5** strand and that of **2** with **4** or **5**.

In the cases of the complexes of XL-DNA with the mismatched sequences, the T_m values of the XL-DNA complexes had large differences in both the enthalpy and entropy changes from those with the full-matched sequence. The large changes in the enthalpy and entropy terms were clearly observed as compared to those of the XL-DNA complex with the full-matched sequence. The changes in the enthalpy term may be attributed to the significant decrease in the hydrogen bondings around the position where the mismatched base was introduced. In addition, the changes in the entropy term may be attributed to the disordered structure of the duplex around the mismatched position and adjacent cross-linker-attached phosphoramidate. Also, a significant difference in the entropy and enthalpy changes between the complexes containing the A- and B-diastereomers with a mismatch base was observed. The large difference in the enthalpy and entropy changes of the **1A** complexes as compared to those of the **1B** ones may indicate that the cross-linker of the A-diastereomer (S_p-configuration) imposes a greater steric stress on the complexes than that of the B-diastereomer (R_p-configuration) because the orientations of these cross-linkers directly depend on the diastereochmistry of the phosphoramidate.[14]

A decrease in the stability of the complexes was observed by further introduction of mismatched bases to the counterpart DNA strands (Table 2). The complexes of **1B** were more stable than those of **1A** in both cases with the strands **4** and **5** having two and three mismatched bases, respectively, indicating that the diastereochemistry is an important factor for the complex formation, and

Table 1 Thermodynamic parameters of the complex formation of the XL-DNA and the counterpart strands containing matched and mismatched bases[a,b,c]

DNA	Counterpart strand **3**-TX	$\Delta H°$/kJ mol^{-1}	$\Delta S°$/kJ mol^{-1}K^{-1}	$\Delta G°$ (298 K)/kJ mol^{-1}	T_m/°C[18]	ΔT_{m1}/°C[b]	ΔT_{m2}/°C[c]
1A	**3**-TA full-match	−510	−1.59	−36.2	48.5	—	−22.9
1A	**3**-TC mismatch	−327	−1.05	−14.1	39.3	−9.2	−28.4
1A	**3**-TG mismatch	−305	−0.98	−13.0	37.2	−10.3	−27.2
1A	**3**-TT mismatch	−335	−1.08	−13.2	39.0	−9.5	−25.6
1B	**3**-TA full-match	−511	−1.57	−43.1	52.9	—	−18.5
1B	**3**-TC mismatch	−414	−1.30	−26.6	44.6	−8.3	−23.1
1B	**3**-TG mismatch	−395	−1.25	−22.5	42.9	−10.0	−21.5
1B	**3**-TT mismatch	−424	−1.34	−24.7	44.6	−8.3	−20.0
2	**3**-TA full-match	−522	−1.51	−72.0	71.4	—	—
2	**3**-TC mismatch	−501	−1.47	−62.9	67.7	−3.7	—
2	**3**-TG mismatch	−505	−1.50	−58.0	64.4	−7.0	—
2	**3**-TT mismatch	−504	−1.49	−59.4	64.6	−6.8	—

[a] The method for acquisition of the thermodynamic parameters is described in ref. 18–21. [b] The ΔT_{m1} values are the difference between the complexes of **1A**, **1B**, and **2** with **3**-TA full-matched strand and those with **3** mismatched strands. [c] The ΔT_{m2} values are the difference between the complexes of **2/3**-TX and those of the XL-DNA with the coresponding counterpart **3**-TX strands.

the strain from the cross-linker strongly affected the stability of the complexes in all the experimental runs.

In summary, the significant difference in the stability between the complexes of XL-DNA with full-matched and mismatched sequences is attributed to structural flexibility of the XL-DNA molecules. Also the strain caused by the cross-linker may induce the disordered duplex structures around the mismatched position and cross-linker, which is dependent on the diastereochemistry of the phosphoramidate. Therefore, the novel strategy utilizing the structural change of the non-canonical DNA complexes containing a branched DNA could be one of the eligible candidates for detecting a mismatch base in a target strand.

This work has been partly supported by a Grant-in-Aid for Scientific Research (Project 17105005, Priority Area (417), 21st Century COE Research, and others) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of the Japanese Government.

Notes and references

1 C. Mao, W. Sun, Z. Shen and N. C. Seeman, *Nature*, 1999, **397**, 144.
2 B. Yurke, A. J. Turberfield, A. P. Mills, Jr, F. C. Simmel and F. C. Neumann, *Nature*, 2000, **406**, 605.
3 H. Yan, X. Ahang, Z. Shen and N. C. Seeman, *Nature*, 2002, **415**, 62.
4 L. Feng, S. H. Park, J. H. Reif and H. Yan, *Angew. Chem., Int. Ed.*, 2003, **42**, 4342.
5 S. Liao and N. C. Seeman, *Science*, 2004, **306**, 2072.
6 W. U. Dittmer, A. Reuter and F. C. Simmel, *Angew. Chem., Int. Ed.*, 2004, **43**, 3550.
7 N. C. Seeman, *Trends Biochem. Sci.*, 2005, **30**, 119.
8 W. Tan, X. Fang, J. Li and X. Liu, *Chem.-Eur. J.*, 2000, **6**, 1107.
9 W. Tan, K. Wang and T. J. Drake, *Curr. Opin. Chem. Biol.*, 2004, **8**, 547.
10 M. Endo and T. Majima, *J. Am. Chem. Soc.*, 2003, **125**, 13654.
11 M. Endo and T. Majima, *Angew. Chem., Int. Ed.*, 2003, **42**, 5744.
12 M. Endo and T. Majima, *Chem. Commun.*, 2004, 1308.
13 M. Endo, S. Uegaki and T. Majima, *Chem. Commun.*, 2005, 3153.
14 M. Endo and T. Majima, *Org. Biomol. Chem.*, 2005, **3**, 3476.
15 The diastereochemistry of an oligonucleotide containing a phosphoramidate unit was examined by enzymatic digestion using alkaline phosphatase and phosphodiesterase (snake venom). The modified DNA was digested into four nucleosides and one phosphoramidate dAp[NH(CH$_2$)$_2$SH]T which has a different retention time on HPLC between the S_p- and R_p-configurations. Therefore, the diastereochemistry can be easily checked by comparing with the phosphoramidates from the digested samples of XL-DNA in ref. 14.
16 MALDI-TOF-MS (negative mode): XL-DNA **1A** and **1B** ($C_{199}H_{253}N_{83}O_{114}P_{18}S_2$); calcd. 6249.1 [M-H], obsd. **1A**: 6249.5, **1B**: 6249.1.
17 Samples (15 μL) containing DNA (0.2 nmol), 10 mM Tris-HCl (pH 7.6), and 0.1 M NaCl were annealed by heating at 90 °C and then cooling to 15 °C at the rate of −1.0 °C min^{-1} using a thermal cycler. Non-denaturing PAGE (20%) was performed at rt, and the gel was visualized by ethidium bromide staining.
18 The T_m values were obtained in solutions containing 2 μM DNA, 10 mM Tris-HCl (pH 7.6), and 0.1 M NaCl.
19 D. D. Albergo, L. A. Marky, K. J. Breslauer and D. H. Turner, *Biochemistry*, 1981, **20**, 1409.
20 M. Petersheim and D. H. Turner, *Biochemistry*, 1983, **22**, 256.
21 Calculation of the thermodynamic parameters was carried out using the van't Hoff plot. Measurements of the T_m values were performed in a solution containing 10 mM Tris-HCl (pH 7.6) and 0.1 M NaCl by increasing the temperature at the rate of 1.0 °C min^{-1}. The concentrations of DNA for the T_m measurements were 0.75, 1, 1.5, and 2.0 μM. The enthalpy (ΔH°) and entropy (ΔS°) changes were calculated using the equation: $T_m{}^{-1} = (2.30R/\Delta H^\circ)\log(C_t/4) + (\Delta S^\circ/\Delta H^\circ)$; ($C_t$ = total concentration of the DNA strands, R = gas constant). All the R^2 values representing the linearity of the four plots were > 0.985.