Thermodynamics and kinetics for base pair opening in the DNA decamer duplexes containing cyclobutane pyrimidine dimer

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1. Introduction

The cis–syn cyclobutane pyrimidine dimer (CPD) (Fig. 1A) is one of the major classes of cytotoxic, mutagenic and carcinogenic UV-induced DNA photoproducts [1,2]. CPD damage is repaired by a variety of DNA repair enzymes [3–5]. In mammalian cells, nucleotide excision repair (NER) is the major pathway for removal of CPD-damaged DNA [2,6]. For bulky DNA lesions, the NER is initiated by binding of XPC-hHR23B to the site of DNA damage [7,8]. However, CPD lesions are recognized poorly by XPC-hHR23B and other damage recognition proteins [8]. This CPD lesion, if left unrepaired, interferes strongly with DNA replication and mutations are introduced at the lesion site with frequencies of 2% and 6% opposite 5′-T and 3′-T of the CPD lesion [9]. Interestingly, when CPD lesions have double T-G mismatches, the binding affinity of the CPD lesion by XPC-hHR23B is dramatically increased [8]. Furthermore, artificial bubble structures have been shown to be recognized efficiently by XPC-hHR23B [8]. Previous structural studies have revealed that double T-G wobble base pairs in a CPD lesion cause severe helical distortion, whereas DNA duplexes that contain perfectly matched base pair or a single 3′-T-G mismatch at CPD lesion sites display little conformational distortion [10,11]. The co-crystal structure of Rad4 – the yeast homologue of mammalian XPC – bound to DNA that contained a CPD lesion base paired with T residues showed that Rad4 binding causes the two damaged base pairs to flip out of the double helix [12]. Unique dynamic features of these CPD-containing DNA duplexes have been predicted on the basis of structural information. However, thermodynamic and kinetic studies of damaged DNA duplexes recognized by XPC-hHR23B have not yet been performed and are needed to understand the damage recognition mechanism of XPC-hHR23B.

In order to address more directly the change in stability of DNA duplexes upon the introduction of T-G mismatches in a CPD lesion, we measured the hydrogen exchange rate constants ($k_{ex}$) of imino protons in DNA duplexes that contained (i) matched (CPD/AA), a single 3′-T-G (CPD/GA), or a double T-G mismatched CPD lesion (CPD/GG); (ii) matched base pairs (TT/AA), a single 3′-T-G mismatch (TT/GA), or a double T-G mismatch (TT/GG) with no CPD lesion (see Fig. 1B) as a function of TRIS concentration at 15°C. These data showed that the double T-G wobble pairs in a CPD lesion significantly destabilized their neighboring base pairs and induce the formation of flexible structures in a DNA duplex. The unique thermodynamic features provide insights into the DNA recognition mechanism for XPC-hHR23B.

2. Materials and methods

The DNA oligonucleotides were purchased from M-biotech Inc. (Seoul, Korea). DNA oligomers were purified by reverse-phase C-18
HPLC and desalted on a Sephadex G-25 column. The CPD-damaged DNA was prepared by direct 254-nm UV irradiation of normal DNA in an aqueous solution and purified as previously described [10,13]. The six DNA duplexes were prepared by dissolving the main strands and the complementary strands at a 1:1 stoichiometric ratio in a 90% H2O/10% D2O aqueous solution containing 10 mM TRIS-d11 (pH 8.5 at 24 \degree C) and 100 mM NaCl. All NMR experiments were performed on a Varian Inova 600 MHz spectrometer (KAIST, Daejeon) using a HCN triple-resonance probe. The exchange rates of the imino protons were determined as previously described [14]. Briefly, the imino hydrogen exchange rate constant ($k_{ex}$) was determined by fitting the data to Eq. (1):

$$\frac{I_0 - I(t)}{I_0} = 2 \frac{k_{ex}}{(R_{1w} - R_{1a})} (e^{-R_{1a}t} - e^{-R_{1w}t})$$

where $I_0$ and $I(t)$ are the peak intensities of the imino proton in the water magnetization transfer experiments at times zero and $t$, respectively, and $R_{1a}$ and $R_{1w}$ are the apparent longitudinal relaxation rate constants for the imino proton and water, respectively, measured in semi-selective inversion recovery 1D NMR experiments [14,15]. The formalism of TRIS-catalyzed proton exchange has been previously described in detail in the Supplementary material and is briefly presented here. The exchange time for the base paired imino proton, $\tau_{ex}$ (inverse of $k_{ex}$), is represented by:

$$\tau_{ex} = \tau_0 + \frac{1}{2k_{op}} \left( \frac{1}{k_{TRIS}} + \frac{1}{k_{int}} \right)$$

where $\tau_0$ is the base pair life time (inverse of opening rate constant, $k_{op}$), and $2k_{op} (=2k_{op}/k_{cl})$ is the apparent equilibrium constant for base pair opening. Curve fitting the $\tau_{ex}$ values of the imino protons as a function of the inverse of [TRIS] with Eq. (2) gives the $2k_{op}$, $\tau_0$ (=1/$k_{op}$), and $k_{int}$ values.

3. Results and discussion

3.1. Hydrogen exchange experiments for imino protons

All NMR experiments were conducted at 15 \degree C because the DNA duplexes that contain both the CPD lesion and G-T wobble pair are far more unstable than normal DNA duplexes [11]. 1D imino proton spectra of the six DNA duplexes studied here at 15 \degree C are shown in Fig. 2. Resonance assignments for the imino protons were made by the analysis of NOESY spectra. The $k_{ex}$ were determined from water magnetization transfer experiments on the imino protons for the DNA duplexes at 15 \degree C. The formation of a CPD lesion slightly increased the $k_{ex}$ of the imino protons of both the CPD lesion and neighboring A-T base pairs. As expected, relatively rapid exchange is observed for the imino protons in the single T-G wobble pair of the TT/GA duplex ($k_{ex} = 209$ and $87$ s$^{-1}$ for T6 and G15). However, the corresponding imino protons in the CPD/GA duplex had significantly smaller $k_{ex}$ values ($k_{ex} = 31$ and $19$ s$^{-1}$ for T6 and G15). The T and G imino protons of the double T-G wobble pairs in both the TT/GG and CPD/GG duplexes exchanged too fast with solvent to be observed in the 1D NMR spectra (Fig. 2). The T14 and T17 imino protons that are positioned next to a CPD lesion with a double T-G mismatch have $k_{ex}$ values of 44 and 36 s$^{-1}$, respectively, whereas these imino protons in the other DNA duplexes have $k_{ex}$ values from 1 to 17 s$^{-1}$ (Table 3S). These results indicate that double T-G wobble pairs in a CPD lesion significantly destabilize their neighboring A-T base pairs, compared to other DNA duplexes (see Fig. 3).

![Fig. 2. 1D imino proton spectra for the (A) TT/AA, (B) TT/GA, (C) TT/GG, (D) CPD/AA, (E) CPD/GA, and (F) CPD/GG duplexes in a 90% H2O/10% D2O solution containing 10 mM TRIS-d11 (pH 8.79) and 100 mM NaCl at 15 \degree C.](image-url)
3.2. Base pair opening dynamics for the DNA duplexes using TRIS as a base catalyst

The $k_{ex}$ for each imino proton in these DNA duplexes was measured at 15 °C as a function of the TRIS concentration (Fig. 4). From these data, the $aK_{op}$, $T_0$ ($=1/k_{op}$), and $k_{int}$ for the C3-G18 and C8-G13 base pairs were determined by curve fitting using Eq. (2). However, in the cases of the A-T base pairs, the only $aK_{op}$ values were determined because of their relatively fast exchanging behaviors. The CPD formation at the T5–T6 site showed little effects on the stabilities of the own base pairs (Fig. 4C) but leads to 2–4-fold larger $aK_{op}$ for the neighboring A4-C17 and A7-C14 base pairs (Table 1). The $aK_{op}$ for T-G wobble pairs could not be determined precisely because of the severe line-broadening of their imino protons, indicating that the T-G wobble pair is much less stable than a non-terminal Watson–Crick T-A base pair under our experimental conditions. The T-G wobble pairs also affect the stabilities of neighboring base pairs. The T5-A16 and A7-T14 base pairs in the TT/GA duplex have 4–6-fold larger values than those of the TT/AA duplex (Table 1). The similar effects were also observed in the CPD-damaged or double T-G mismatched DNA duplexes (Table 1).

Surprisingly, in the CPD/GG duplex, the $k_{ex}$ values of the G13 and G18 imino protons showed a larger dependence on TRIS concentration than did the corresponding $k_{ex}$ values of other duplex DNAs (Fig. 4); indeed, the $aK_{op}$ for the C3-G18 and C8-G13 base pairs were 11- and 17-fold larger, respectively, than those of the TT/AA duplex. According to these base pair stabilities, unlike properly matched or single-matched CPD lesions, double T-G mismatches in a CPD-containing DNA duplex could induce the formation of a flexible structure in this double helix like bubble structure, in which six consecutive base pairs are remarkably unstable.

3.3. Gibbs free energy analysis

The Gibbs free energy for the thermal stability of each base pair ($\Delta G_{bp}^0$) can be calculated from $aK_{op}$ by Eq. (3) shown below:

![Fig. 3. The relative peak intensities in the difference spectra, $|I_0 - I(t)|/I_0$, as a function of delay time for the (A) T17, (B) T5, and (C) T14 imino protons in the TT/AA (open circle), TT/GA (open triangle), TT/GG (open square), CPD/AA (closed circle), CPD/GA (closed triangle), and CPD/GG (closed square) duplexes at 15 °C. Solid lines indicate the best fitting of these data using Eq. (1).

![Fig. 4. The hydrogen exchange times ($t_{ex}$) of the (A) G18, (B) T17, (C) T5, (D) G13, (E) T14, and (F) T6 imino protons of the TT/AA (open circle), TT/GA (open triangle), TT/GG (open square), CPD/AA (closed circle), CPD/GA (closed triangle), and CPD/GG (closed square) duplexes as a function of the inverse of TRIS concentration at 15 °C. Solid lines indicate the best fitting of these data using Eq. (2).]
Subsequently, we extended our focus on base pair opening kinetics of each base pair in the various DNA duplexes by comparing Watson–Crick base pairs with two adenine bases, as evident from the small Gibbs free energy differences (ΔGbp = 1.03 and 1.61 kcal/mol for T5/C1 and T5/G13 base pairs in the CPD/GG duplex, respectively).

Table 2

<table>
<thead>
<tr>
<th>Base pair</th>
<th>TT/AA</th>
<th>TT/GG</th>
<th>CPD/AA</th>
<th>CPD/GA</th>
<th>CPD/GG</th>
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<td>C3 G18</td>
<td>0.2</td>
<td>0.4</td>
<td>0.1</td>
<td>0.2</td>
<td>1.4</td>
</tr>
<tr>
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<td>0.3</td>
<td>1.1</td>
<td>0.7</td>
<td>0.9</td>
<td>1.8</td>
</tr>
<tr>
<td>T5 X16b</td>
<td>1.0</td>
<td>&gt;1.9</td>
<td>0.2</td>
<td>&gt;1.0</td>
<td>&gt;1.9</td>
</tr>
<tr>
<td>T6 X15b</td>
<td>0.3</td>
<td>&gt;2.3</td>
<td>0.7</td>
<td>&gt;2.3</td>
<td>&gt;2.3</td>
</tr>
<tr>
<td>A7 T14</td>
<td>0.8</td>
<td>1.3</td>
<td>0.5</td>
<td>0.7</td>
<td>1.9</td>
</tr>
<tr>
<td>C8 G13</td>
<td>0.6</td>
<td>1.0</td>
<td>0.4</td>
<td>0.6</td>
<td>1.6</td>
</tr>
</tbody>
</table>

ΔGbp = −RT ln(κbp)

We quantitatively measured the thermal stability and opening kinetics of each base pair in the various DNA duplexes by comparing the ΔGbp values of the next neighboring base pairs, as evident from the small Gibbs free energy differences when compared with a normal DNA duplex (ΔGbp = 0.18 kcal/mol for CPD/AA, Table 2). When the DNA duplex contained a single T–G wobble pair in a CPD (CPD/GA duplex), it displayed significantly higher Gibbs free energy differences (ΔGbp = 1.03 and 1.61 kcal/mol for T5–A16 and T6–A15 base pairs, respectively). Similar values were obtained for TT/GG duplex (Table 2). The ΔGbp values for two T–G wobble pairs in both TT/GG and CPD/GG duplexes could not be determined because of the severe line-broadening of imino proton resonances.

Surprisingly, the ΔGbp values of the next neighboring base pairs such as C3–G18 and C8–G13 base pairs in the CPD/GG duplex were also significantly high (1.37 and 1.64 kcal/mol, respectively), whereas the corresponding values in the other duplexes were less than 0.95 kcal/mol.

3.4. Implications for DNA damage recognition by the XPC-hHR23B complex

Structural study of matched and mismatched CPD-containing DNA duplexes has suggested that, during NER, the XPC-hHR23B complex recognizes DNA damage by direct searching for conformational distortions, such as a flexible backbone, a bent helix, or an unusual groove width [11]. This mechanism accounts for the fact that the (6–4) photoproduct, which causes severe helical distortion [16], is the best DNA substrate for the XPC-hHR23B complex. According to the co-crystal structure of the Rad4–Rad23–CPD–damaged DNA complex [12], the CPD base pairs might be flipped out of the helix after recognition by XPC-hHR23B, the homologues of Rad4–Rad23. This structural feature explains the observed direct correlation between the binding affinities of XPC-hHR23B for the lesion sites and the thermal stability of the DNA duplex [11]. Our study quantitatively measured the thermal stability and opening kinetics of each base pair in the various DNA duplexes. The bases that are part of the CPD formed stable Watson–Crick base pairs with two adenine bases, as evident from the small Gibbs free energy differences when compared with a normal DNA duplex (ΔGbp = 0.18 kcal/mol for CPD/AA, Table 2). When the DNA duplex contained a single T–G wobble pair in a CPD (CPD/GA duplex), it displayed significantly higher Gibbs free energy differences (ΔGbp = 1.03 and 1.61 kcal/mol for T5–A16 and T6–A15 base pairs, respectively). Similar values were obtained for TT/GG duplex (Table 2). The ΔGbp values for two T–G wobble pairs in both TT/GG and CPD/GG duplexes could not be determined because of the severe line-broadening of imino proton resonances.

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On the basis of our results, the matched or single mismatched CPD lesion site exists in equilibrium between the closed and the CPD flip out states. Intriguingly, a double mismatch at the CPD lesion facilitates opening of the neighboring base pairs as well as
CPD lesion site itself. Subsequently, a flexible structure develops in the DNA helix around the CPD lesion, and this unique structure can considerably increase the binding affinity of XPC-hHR23B for a DNA duplex. This possible mechanism can successfully explain the high binding affinity of XPC-hHR23B for both a double mismatched CPD lesion and an artificial small bubble structure in a DNA duplex [8].

Acknowledgements

This work supported by a Grant from the National Creative Research Initiative Program (to B.-S.C.); an EB-NCRC Grant (R15-2003-012-01001-0); the KOSEF Grant (R01-2007-000-10691-0 to J.-H.L.); the KRF Grant (KRF-2008-331-C00178 to J.-H.L.), funded by the Korean Government (MEST). This study made use of the NMR facility at Korea Basic Science Institute, which is supported by Bio NMR Research Program of the Korean Ministry of Science and Technology (E28070).

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2009.05.015.

References