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

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ABSTRACT

The cyclobutane pyrimidine dimer (CPD) is one of the major classes of cytotoxic and carcinogenic DNA photoproducts induced by UV light. Hydrogen exchange rates of the imino protons were measured for various CPD-containing DNA duplexes to better understand the mechanism for CPD recognition by XPC-hHR23B. The results here revealed that double T·G mismatches in a CPD lesion significantly destabilized six consecutive base pairs compared to other DNA duplexes. This flexibility in a DNA duplex caused at the CPD lesions with double T·G mismatches might be the key factor for damage recognition by XPC-hHR23B.

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

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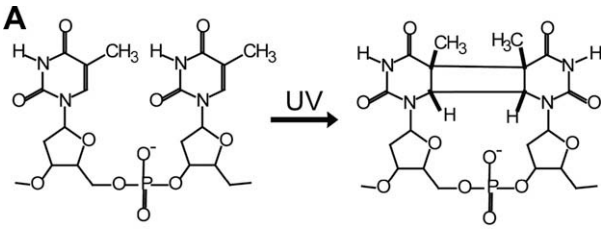

B

1 2 3 4 5 6 7 8 9 10
5'- C G C A T = T A C G C -3'
3'- G C G T Y X T G C G -5'
20 19 18 17 16 15 14 13 12 11

T=T	X	Y	duplex
TpT	A	A	TT/AA
T[CPD]T	A	A	CPD/AA
TpT	G	A	TT/GA
T[CPD]T	G	A	CPD/GA
TpT	G	G	TT/GG
T[CPD]T	G	G	CPD/GG

Fig. 1. (A) The chemical structure of the CPD lesion. (B) DNA sequence contexts of the decamer DNA duplexes studied here.

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% H_2O/10% D_2O aqueous solution containing 10 mM TRIS-d_{11} (pH 8.5 at 24.2 °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):

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

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, τ_{ex} (inverse of k_{ex}), is represented by:

$$\tau_{ex} = \tau_0 + \frac{1}{\alpha K_{op}}\frac{1}{(k_i[TRIS] + k_{int})} \quad (2)$$

where τ_0 is the base pair life time (inverse of opening rate constant, k_{op}), and αK_{op} ($=\alpha k_{op}/k_{cl}$) is the apparent equilibrium constant for base pair opening. Curve fitting the τ_{ex} values of the imino protons as a function of the inverse of [TRIS] with Eq. (2) gives the αK_{op}, τ_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 °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 °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 °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).

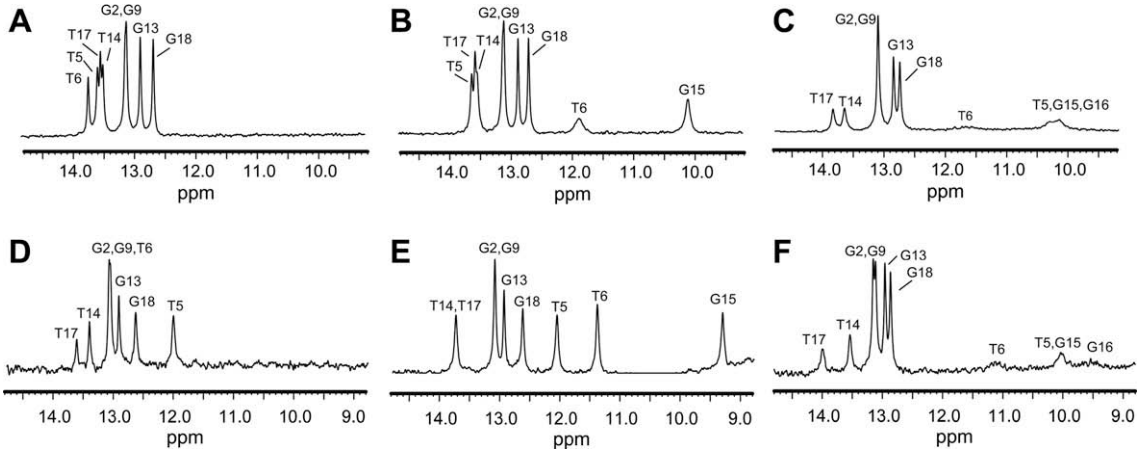

Fig. 2. 1D imino proton spectra for the (A) TT/AA, (B) TT/GA, (C) TT/GG, (D) CPD/AA, (E) CPD/GA, and (F) CPD/GG duplexes in a 90% H_2O/10% D_2O solution containing 10 mM TRIS-d_{11} (pH 8.79) and 100 mM NaCl at 15 °C.