Effect of T·G and T·T Mismatches of *cis-syn* Cyclobutane Pyrimidine Dimer on the Thermal Stabilities of DNA Decamer Duplexes

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The *cis-syn* cyclobutane pyrimidine dimer (CPD) (Fig. 1A) is one of the major classes of cytotoxic, mutagenic and carcinogenic DNA photoproducts induced by ultraviolet (UV) irradiation.[1,2] Nucleotide excision repair (NER) is the major pathway for removal of CPD lesion in mammalian cells.[3] The initiation step of the NER pathway is the recognition of a single DNA damage among an extensive background of undamaged DNA.[4] XPC-hHR23B is thought to be the primary damage recognition protein complex in the human NER pathway.[5,6] XPC-hHR23B binds preferentially to bubble structure and bulky DNA but poorly recognizes CPD damaged DNA.[5,7] However, the presence of double T·G mismatches of the CPD lesion significantly enhances XPC-hHR23B binding whereas a single mismatched CPD shows marginally detectable binding activity.[7] NMR study suggested that the enhancement of XPC-hHR23B binding affinity to CPD results from the helical distortion caused by the double T·G mismatches of the CPD.[8] It was also suggested that the helical instability of the CPD damaged DNA duplex containing its double T·G mismatches is one of the important factor for the damage recognition by XPC-hHR23B.[8]

In order to understand the molecular mechanism of the XPC-hHR23B binding to CPD lesion, we performed the thermodynamics studies on the CPD damaged DNA duplexes containing the matched T·A base pair (CPD/AA) or mismatched T·G base pairs (CPD/GA or CPD/GG) or T·A base pair (CPD/TA) at the lesion site (see Fig. 1B). The thermodynamics studies on the normal DNA duplexes (TT/AA, TT/GA, TT/GG, and TT/TA) containing the same sequences described above (see Fig. 1B) were also performed to provide the mismatch effect on the helical stability as control data.

Experimental Section

The DNA decamer duplexes, 5'-CGCATTACGC-3' (TT-10), 5'-GCGTAATGCG-3' (AA-10), 5'-GCGTGATGCG-3'

(GA-10), 5'-GCGTGGTGCG-3' (GG-10), 5'-GCGTTATGCG-3' (TA-10), were purchased from Genotech Inc. (Daejeon, Korea) or IDT Inc. (Seoul, Korea). DNA oligmers were purified by reverse-phase C-18 HPLC and desalted by Sephadex G-25 column.[9] The CPD-damaged TT-10 DNA decamer (CPD-10) was prepared by direct 254-nm UV irradiation of a TT-10 oligomer in aqueous solution and purified as described.[8,10] The eight DNA duplexes were prepared by dissolving the main strands (TT-10 or CPD-10) and the complementary strands (AA-10, GA-10, GG-10, or TA-10) at a 1:1 stoichiometric ratio in an aqueous solution containing 10 mM Tris-d$_{11}$ (pH 8.5) and 100 mM NaCl.

The DNA absorbance was recorded on Shimadzu UV2401PC UV/Vis spectrophotometer equipped with
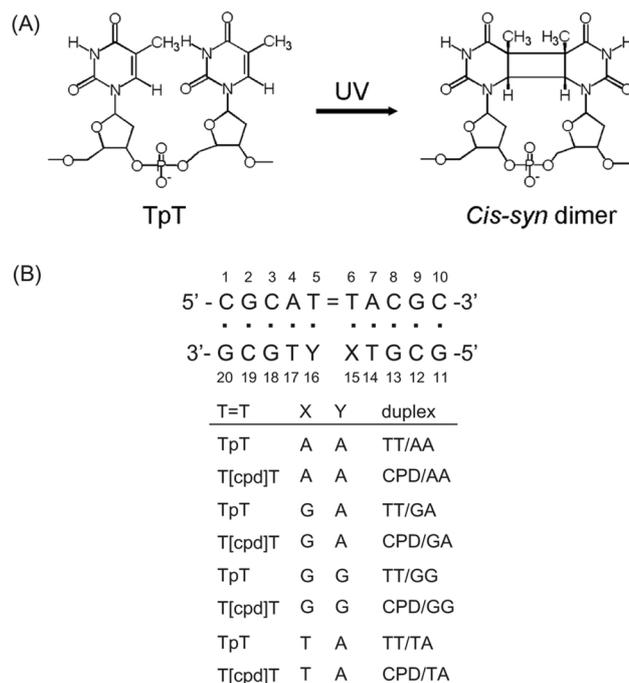
Figure 1. (A) The chemical structure of the CPD lesion (B) DNA sequence contexts of the DNA duplexes studied here.

Results and Discussion
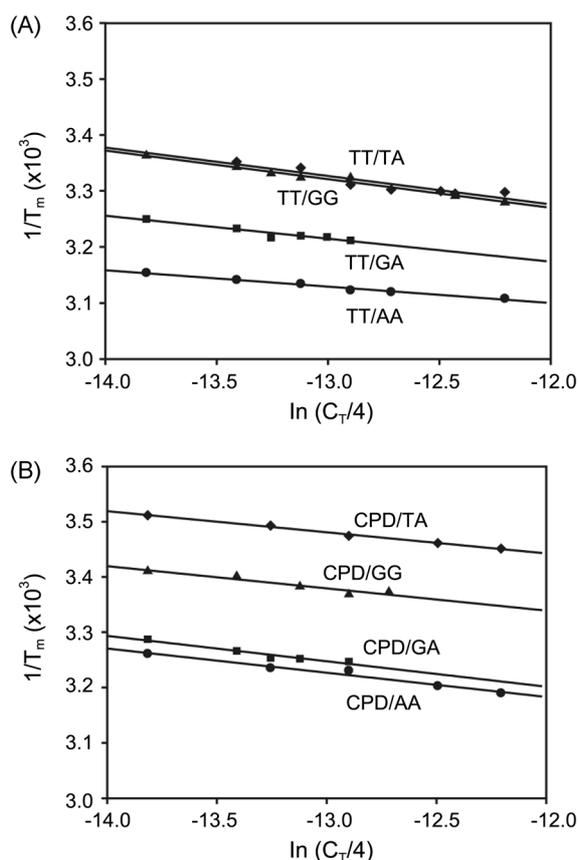
Figure 2. The van't Hoff plots ($1/T_m$ *vs* $\ln(C_T/4)$) for the (A) TT-containing DNA duplexes and (B) CPD-containing DNA duplexes in the aqueous solution containing 10 mM Tris-d_{11} (pH 8.5) and 100 mM NaCl. The lines drawn represent the least square fits.

thermoelectric cell holder. The thermal denaturation of each DNA duplexes was monitored simultaneously at 265-, 275-, and 320-nm. The temperature was ramped from 5 to 80 °C at 0.1 °C/min controlled by temperature controlling program and the DNA absorbance was recorded every 0.1 °C. The melting temperature (T_m) of the thermal denaturation was obtained by the average method using T_m analysis program on the absorbance at 265- and 275-nm which were subtracted by A_{320nm} values and then their average values were used in this study.

Plots of $1/T_m$ *vs* $\ln(C_T/4)$ where C_T is the total strand concentration ranging from 4- to 20-μM were constructed (Fig. 2) in order to determine the thermodynamic parameter (ΔH^o and ΔS^o). For non-self-complementary strands, the relationship of the duplex melting T_m and DNA concentration[11,12] is expressed as

$$1/T_m = (R/\Delta H^o)\ln(C_T/4) + \Delta S^o/\Delta H^o \quad (1)$$

The free energies of the duplex denaturation are calculated by the standard Gibb's equation expressed as

$$\Delta G^o = \Delta H^o - T\Delta S^o \quad (2)$$

Thermodynamic parameters for eight DNA duplexes are listed in Table 1. At 5 μM concentration of DNA duplexes, the T_m of the TT/GA was 8.9 °C lower than that of the TT/AA duplex (Table 1) and thus the $\Delta G^o{}_{298K}$ of the TT/GA duplex which was calculated at 25 °C is 2.55 kcal/mol larger than that of the TT/AA duplex (Table 1). This result indicates that the change from T·A Watson-Crick base pair to T·G wobble pair lead to the thermal instability of the DNA duplex decamer with 2.55 kcal/mol larger ΔG^o at 25 °C. The $\Delta G^o{}_{298K}$ of the TT/GG duplex is 1.6 and 4.2 kcal/mol larger than those of the TT/GA and TT/AA duplexes, respectively (Table 1), indicating that the effects of the double T·G wobble pairs at central TT site are additive on the thermal stability of DNA duplex. Interestingly, it was observed that the T·T wobble pair remarkably destabilizes the DNA duplex with 4.25 kcal/mol larger $\Delta G^o{}_{298K}$ which is the same with the destabilization effect of the double T·G wobble pairs.

The T_m of the CPD/AA duplex which has the CPD lesion at central T5-T6 site was 10.6 °C lower than that of the TT/AA duplex (Table 1). Thus, the $\Delta G^o{}_{298K}$ of the CPD/AA duplex is 2.84 kcal/mol larger than that of the TT/AA duplex, indicating that the CPD lesion causes a similar degree of helical instability with the single T·G wobble pair (Table 1). The effects of the mismatches of the CPD lesion on the thermal helical stabilities are quite different from those of normal T-T site (Fig. 2). Surprisingly, the difference in the $\Delta G^o{}_{298K}$ between the CPD/GA and CPD/AA duplexes is only 0.35 kcal/mol contrasting to the effect of single T·G

Table 1. Thermodynamic parameters for the DNA decamer duplexes in the 10 mM Tris-d_{11} (pH 8.5), 100 mM NaCl solution

Duplex	TT	X	Y	ΔH^o (kcal/mol)	ΔS^o (cal/K·mol)	$\Delta G^o{}_{298K}$ (kcal/mol)	$\Delta\Delta G^o{}_{298K}$ (kcal/mol)[a]	T_m (°C)[b]
TT/AA	TpT	A	A	-68.0 ± 1.0	-186.8 ± 3.3	-12.29 ± 0.29	0	47.2
TT/GA	TpT	G	A	-48.5 ± 1.9	-130.1 ± 6.6	-9.74 ± 0.62	2.55	38.3
TT/GG	TpT	G	G	-38.8 ± 0.6	-103.2 ± 2.2	-8.10 ± 0.21	4.19	27.9
TT/TA	TpT	T	A	-39.1 ± 1.7	-104.3 ± 6.5	-8.04 ± 0.62	4.25	29.0
CPD/AA	T[CPD]T	A	A	-45.5 ± 1.1	-121.0 ± 3.8	-9.45 ± 0.37	2.84	36.6
CPD/GA	T[CPD]T	G	A	-43.3 ± 1.5	-114.8 ± 5.3	-9.10 ± 0.52	3.19	35.0
CPD/GG	T[CPD]T	G	G	-49.4 ± 2.1	-141.0 ± 7.5	-7.35 ± 0.50	4.93	23.9
CPD/TA	T[CPD]T	T	A	-51.9 ± 0.6	-154.9 ± 2.0	-5.77 ± 0.10	6.52	14.8

[a]difference between the mismatched duplex and the TT/AA duplex. [b]determined for 5 μM strand concentration

wobble pair at T-T site (2.55 kcal/mol) (Table 1). This result means that the DNA duplex containing the CPD lesion matched or single T·G mismatched has significantly higher thermal stability than the double T·G mismatched DNA duplex. However, the double T·G wobble pairs of the CPD lesion caused significant helical instability with 2.10 kcal/mol higher ΔG^o_{298K} than the CPD/AA duplexes (Table 1). Actually, the DNA duplex containing the double T·G mismatched CPD lesion is unstable with 23.3 ^{o}C lower T_m and 4.93 kcal/mol higher ΔG^o_{298K} than the normal TT/AA DNA duplex. The influence of the double T·G mismatches of the CPD lesion is much greater than that of normal DNA duplex and might be similar to that of three consecutive T·G mismatches in DNA duplex. This greater thermal instability of the CPD/GG duplex contributes the damage recognition of XPC-hHR23B protein via base pair opening process. The CPD/TA duplex is extremely unstable by both effects of the CPD lesion and T·T wobble pair (Table 1), indicating that the T·T mismatch of the CPD lesion might be good substrate for XPC-hHR23B protein.

Our study clearly explains why XPC-hHR23B easily recognizes the double T·G mismatched CPD lesion but rarely does the matched or single T·G mismatched CPD lesions. The single T·G mismatch of CPD lesion maintain normal B-form helix but its double T·G mismatches caused severe helical distortion.[8] In addition, the double T·G mismatches of CPD lesion induced the remarkable helical instability that might contribute to the CPD recognition by XPC-hHR23B. Thus we concluded that the important factors for damage recognition of the XPC-hHR23B are not only helical distortion but also helical instability caused by DNA lesion.

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