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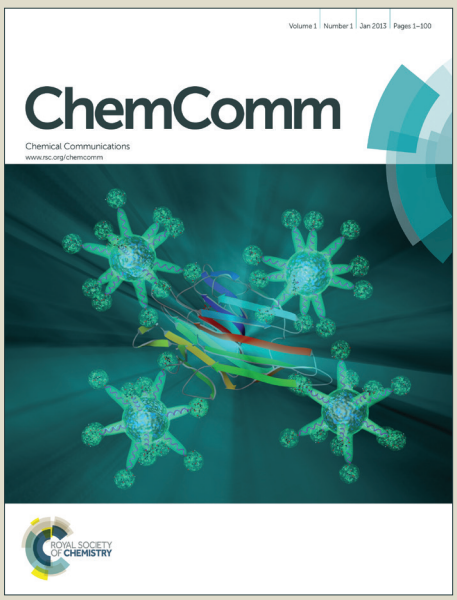

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Deformable nature of various damaged DNA duplexes estimated by an electrochemical analysis on electrodes

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We report bending flexibility of damaged duplexes possessing an apurinic/apyrimidinic (AP) site analogue, a cyclobutane pyrimidine dimer (CPD), and a pyrimidine(6–4)pyrimidone photoproduct (6–4PP). Based on the electrochemical evaluation on electrodes, the duplex flexibilities increased in the following order of the lesions: CPD < AP < 6–4PP. We discussed the possibility that the emerging local flexibility might be a good sign to UV-damaged DNA-binding proteins on duplexes.

UV exposure of DNA is well-known to afford CPDs through [2+2] cycloaddition of two adjacent pyrimidines, especially thymine bases. Additionally, there has been realized for the presence of 6–4PPs via an oxetane intermediate. Cells are equipped with various types of repair enzymes such as DNA glycosylases, photolyases, and AP endonucleases, which recognize the damaged regions in DNA. In the complexes with these proteins, bent structures of the DNA helix are frequently observed.[1] On the other hand, the overall conformations of damaged DNA without the protein binding have been described either as the canonical B-form or as the bent structure, and different types of structures were sometimes reported for the duplexes containing the same lesion.[2] In the case of the 6–4PP, inconsistent structures, with large helix bending[3] and with smaller or no bending,[4] were presented. Recently, selection from dynamic conformational ensembles was proposed for biomolecular recognition,[5] and this mechanism may be applied not only to RNA but also to DNA. To the best of our knowledge, however, little is known about systematic evaluation of the flexibility of damaged double-stranded DNAs (ds-DNAs) compared with those of the corresponding wild-type counterparts.

We have developed a conceptually new discrimination method of genomic variants such as single nucleotide polymorphisms (SNPs) and insertion/deletion mutations by use of electrochemical DNA probes.[6–8] The probes have a redox active ferrocene/isoquinoline-conjugate (FcIq) and a propane thiol linker connected to both the ends of a single-stranded DNA. Having been immobilized on gold electrodes by means of gold-thiol chemistry, the probe was hybridized with target DNAs and scanned by square wave voltammetry (SWV). In this way, we observed the different electrochemical responses from fully-matched and single base-mismatched ds-DNAs for SNPs[7] or bulge-containing ds-DNAs for insertion/deletion mutations.[8] We also applied this strategy to the successful trace of DNA deamination catalyzed by APOBEC3G.[9] The small structural changes resulted from the genomic variants could be read out as an electrochemical response, which proved to highly depend on the Brownian bending motion of the ds-DNA on electrodes against the SWV time scale.[7a,10] Herein, we report the electrochemical evaluation of the bending flexibility in ds-DNAs containing CPD and 6–4PP (Figure 1A). In addition, the flexibility of ds-DNAs consisting of an AP analogue (AP^{THF}) that has a tetrahydrofuran (THF) unit was measured and compared because the damaged ds-DNAs showed almost the same kinked structure with

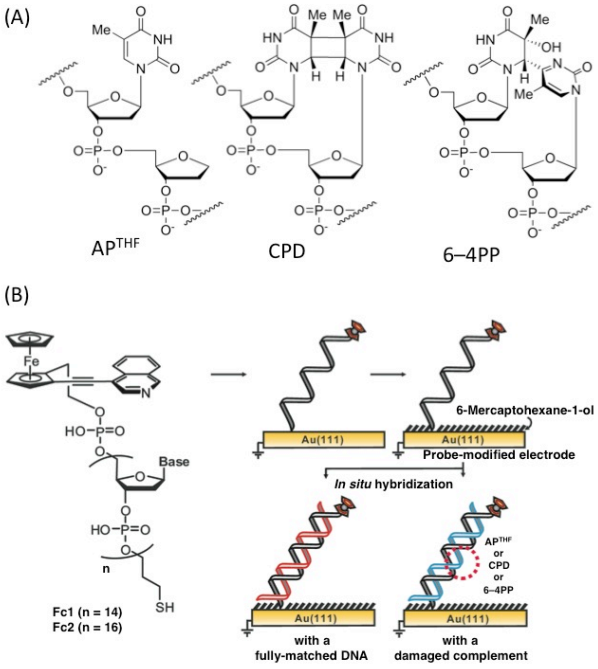

Figure 1. (A) Chemical structures of AP^{THF}, CPD, and 6–4PP moieties in DNA. (B) Schematic illustration of the duplex formation of the electrochemical DNA probe **Fc** with fully-matched and damaged complements containing the AP^{THF}, CPD, and 6–4PP.

those containing CPD and 6–4PP in the DNA-protein complexes.[1b,c] We also discuss the possible role of the local flexibility to DNA-protein complexation.

Synthesis of electrochemical probes[6] (**Fc1** and **Fc2**) and photo-damaged complements[11] (**CPD$_{14}$**, **6-4PP$_{14}$**, **CPD$_{16}$**, and **6–4PP$_{16}$**) was performed in a manner similar to those previously reported (see Table 1 for the sequences). In advance, we identified the firm duplex formations even with the damaged complements in order to rule out the ambiguity of insufficient hybridization for accurately evaluating the duplex flexibility. The natural sequences of 14-mer **1** and 16-mer **2** with the same context as the electrochemical probes were examined for duplex formation in a buffer (10 mM sodium cacodylate, 0.5 M NaCl (pH 7.0)). In the 14-mer combinations for **1**, melting temperatures (T_m) of 70, 53, 64, and 47 ºC were determined from UV-melting curves with the complements **Wild$_{14}$**, **AP$_{14}$**, **CPD$_{14}$** and **6–4PP$_{14}$**, respectively. Likewise, in the 16-mer for **2**, T_m values of 73 (**Wild$_{16}$**), 59 (**AP$_{16}$**), 64 (**CPD$_{16}$**), and 54 (**6–4PP$_{16}$**) ºC were obtained (Figure 2). Although all of the damaged combinations showed lower T_m values compared with those of the fully-matched ones, **1** and **2** tightly formed ds-DNAs with every component at 25 ºC. The duplex formation was also confirmed by circular dichroism in the same buffer at 25 ºC. The 14-mer combinations from **Wild$_{14}$** and **6–4PP$_{14}$** that produced the ds-DNA of the lowest T_m value gave almost the same spectra characteristic of a typical ds-DNA (Figure S1 in Electronic Supplementary Information (ESI)).

The electrochemical probes **Fc**s were immobilized onto commercially available gold-electrodes (see Experimental section in ESI). Fully-matched ds-DNAs were formed when the fully complementary **Wild**s were hybridized *in situ* on the probe-modified electrodes, while damaged ds-DNAs with **AP**s, **CPD**s, and **6-4PP**s (Figure 1B). To estimate the bending flexibility of the 14-mer ds-DNAs, cyclic voltammetry (CV) measurements were carried out at various scan rates (v) for the **Fc1**-based ds-DNAs from **Wild$_{14}$**, **AP$_{14}$**, **CPD$_{14}$**, and **6–4PP$_{14}$**. In all the cases, reversible electrochemical peaks were observed around +0.27 V (Ag/AgCl) derived from the oxidation of the Fc/Iq reporter in the probe. The plots for the anodic peak current (i_{pa}) over the square root of the scan rate (v) against log(v) had bell-shaped profiles in each case, demonstrating the elastic bending mechanism for the electrochemical response of the ds-DNAs. The log(v) value that gives rise to a maximum $i_{pa} / v^{1/2}$ depends on the flexibility of the ds-DNAs on electrodes. Thus, more flexible ds-DNAs show larger values, and vice versa. Compared with the log(v) value of the fully-matched **Fc1•Wild$_{14}$**, we found almost the same value of **Fc1•CPD$_{14}$** and the increased ones of **Fc1•AP$_{14}$** and **Fc1•6–4PP$_{14}$** (Figure S2 in ESI).[10] The profiles mean that **Fc1•AP$_{14}$** and **Fc1•6–4PP$_{14}$** are more flexible than **Fc1•Wild$_{14}$** and **Fc1•CPD$_{14}$**.

The flexibility is quantified by use of the rate constant k for the electron-transfer from the FcIq to the electrode according to Laviron's equation for the ΔE_{pa} ($\Delta E_{pa} = E_{pa} - E_{1/2}$) against the log($v$). The rate constant k, directly proportional to its flexibility, of the fully-matched **Fc1•Wild$_{14}$** was determined to be 136 s^{-1}, while 202, 98, and 240 s^{-1} for the damaged **Fc1•AP$_{14}$**, **Fc1•CPD$_{14}$**, and **Fc1•6–4$_{14}$**, respectively (Figure S3 in ESI).[12] Figure 2A summarizes these and some additional k values obtained from the single-base mismatched **Fc1•AT$_{14}$** and the adjacent two-base mismatched **Fc1•AA$_{14}$**. The data suggest that the duplex flexibility increased in the following order of the 14-mer complements: **Wild$_{14}$** ≈ **CPD$_{14}$** < **AP$_{14}$** (≈ single-base mismatched **AT$_{14}$**) < **6–4PP$_{14}$** (≈ adjacent double-base mismatched **AA$_{14}$**). CPD has a doubly-bridged condensation structure of the two adjacent thymine rings to produce a nearly parallel hydrogen-bonding array compatible with the original state. This nucleotide-level situation would make the CPD-containing ds-DNA be similar to the fully-matched one, so that the duplex remained stiff. Since AP lacks single base within the ds-DNA, it seems to be reasonable that the AP^{THF}-containing and the single-base mismatched ds-DNAs resemble each other in terms of their flexibility. This phenomenon was also seen between the 6-4PP-containing and the adjacent two-base mismatched ds-DNAs. The chemical structure of the 6–4PP is a perpendicularly connected thymine-thymine dimer that hardly forms a canonical base paring at the two adjacent positions. This may be a reason why the **6–4PP$_{14}$** complement caused its duplex to be so flexible as the two-base mismatched **AA$_{14}$** did.

Table 1. Sequences for the DNA probes and the complements in this study.

abbreviation	sequence
1	3'-GCG CTT AAC GCG GG-5'
2	3'-GTA GCC GAA GCG CGT G-5'
Fc1	3'-HS-$(CH_2)_3$-GCG CTT AAC GCG GG-FcIq-5'
Fc2	3'-HS-$(CH_2)_3$-GTA GCC GAA GCG CGT G-FcIq-5'
Wild$_{14}$	3'-CCC GCG TTA AGC GC-5'
AP$_{14}$	3'-CCC GCG (AP^{THF})TA AGC GC-5'
CPD$_{14}$	3'-CCC GCG (CPD)A AGC GC-5'
6–4PP$_{14}$	3'-CCC GCG (6-4PP)A AGC GC-5'
AT$_{14}$	3'-CCC GCG ATA AGC GC-5'
AA$_{14}$	3'-CCC GCG AAA AGC GC-5'
Wild$_{16}$	3'-CAC GCG CTT CGG CTA C-5'
AP$_{16}$	3'-CAC GCG C(AP^{THF})T CGG CTA C-5'
CPD$_{16}$	3'-CAC GCG C(CPD) CGG CTA C-5'
6–4PP$_{16}$	3'-CAC GCG C(6-4PP) CGG CTA C-5'
AT$_{16}$	3'-CAC GCG CAT CGG CTA C-5'
AA$_{16}$	3'-CAC GCG CAA CGG CTA C-5'

FcIq is a ferrocene/isoquinoline conjugate-modified nucleotide residue. Underlines indicate modified residues from the wild-type sequences. See Figure 1A for the chemical structures of AP^{THF}, CPD, and 6–4PP.

The corresponding k values were collected for the **Fc2**-based 16-mer ds-DNAs that have different sequence context with extra chain length. All of the k values decreased as compared with those in the 14-mers (Figure 2B). The electron transfer must be attained by the FcIq redox head being moved close to electrode surface by the elastic diffusion of the ds-DNAs. Thus, the longer ds-DNAs are likely to delay the redox head for approaching to the surface, while the different sequences of the 14-mer and 16-mer ds-DNAs also certainly play a role in the observed k values. Nevertheless, the trend for the flexibilities of the 16-mer ds-DNAs is roughly similar to that of the 14-mer ds-DNAs. Considering the error bars, we found that CPD-containing duplexes show very close k values to the corresponding wild-type DNAs. Interestingly, the rate constants seem to be inversely proportional to the T_m values both for the 14-mer and 16-mer series,[13] although k relates to the kinetics and T_m to the thermodynamics of the ds-DNAs.

The UV-damaged DNA-binding (UV-DDB) protein specifically binds to ds-DNA containing the AP, the CPD, and the 6–4PP at the first step of the global-genome nucleotide excision repair (GG-NER). X-ray structural analysis revealed that the complexes consisting of the UV-DDB protein and these damaged duplexes displayed a similar binding configuration each other.[1b,c] Upon the formation of the DNA–protein complex, the DDB2 subunit causes a large kink (ca. 40°) at the damage site in DNA, and extrudes the AP^{THF} and the

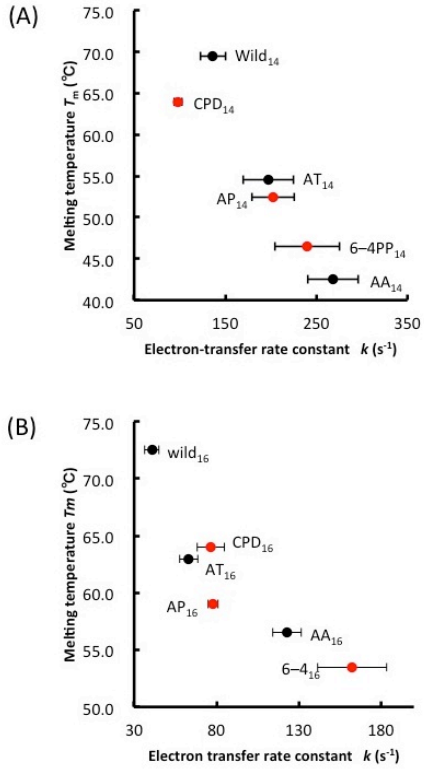

Figure 2. Summary of T_m against k plots for a series of (A) 14-mer and (B) 16-mer ds-DNAs. The T_m values were obtained by use of **1** and **2**. Probes **Fc1** and **Fc2** were used for the determination of k values that were based on three measurements. Error bars indicate the standard deviation (SD).

6–4PP into its binding pocket.[1b] However, prior to the protein binding, the damaged duplex itself showed an overall double-helical conformation without considerable distortions, as revealed by FRET (fluorescent resonance energy transfer) measurements in solution.[4b,14a] Since the UV-DDB protein does not directly recognize the chemical structure of the lesion, we wish to suggest the importance of the deformable flexibility of DNA duplexes for the specific recognition of damage sites by the UV-DDB protein in the GG-NER. The damage-induced local flexibility of the ds-DNA, which was shown in this study, enables the UV-DDB protein to form a distorted DNA–protein complex with less energy. On the other hand, the affinity of the CPD-containing DNA for the UV-DDB protein is known to be lower than those of the duplexes containing the AP^{THF} and the 6–4PP,[14] although a similar kinked DNA structure was observed in the complex with the UV-DDB protein.[1c] Our results showed that the CPD-containing duplexes exhibited less bending flexibility than the AP^{THF}- and especially 6–4PP-containing ones. This observation is in line with the protein binding properties. Human genome DNA incurs over 10,000 damages per cell everyday.[15] Because the genome damages sometimes bring about fatal error in cells, an astronomical number of exact repairs are necessary for all over the body. Thus, it is undoubted that there must be a highly effective and precise searching system against the damaged sites. If repair proteins slide or hop on ds-DNA, the emerging local flexibility may be a good sign for searching damaged sites. Other groups also reported such conjectures in human NER and in mismatch repair.[16]

In conclusion, we systematically evaluated the bending flexibility of the damaged DNA duplexes containing AP^{THF}, CPD, and 6–4PP sites by the CV-based electrochemical method. The 6–4PP-containing duplex was found to be most flexible among them, while the CPD-containing duplex maintained the stiffness of the pre-damaged state. The present study clarified that the local disorder for the base pairing at the damaged site predominantly affects the bending flexibility of the DNA. We also discussed the possibility that the repair enzymes might target the flexibility at the first stage of the DNA-protein complexation. Further study on this topic is now underway in our laboratory.

Notes and references

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† Electronic Supplementary Information (ESI) available: Experimental details and Figures S1–2. See DOI: 10.1039/c000000x/

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