Selective guanine oxidation by UVB-irradiation in telomeric DNA

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The combination of the transient absorption measurement and DNA damage quantification by HPLC clearly demonstrated the preferential excitation of guanine and its decomposition in quadruplex DNA by UVB-irradiation.

Telomeres consist of tandem repeats of short G-rich DNA sequences and form quadruplexes based on the G-quartet structure. It has been determined that the length of the telomeres decreases with every cell division, and the telomere length in cells obtained from tissues *in vivo* is inversely related to the individual's physiological age.[1,2] Hence, telomere shortening has been suggested to be a molecular biomarker of aging. Recently, UVA-irradiation in the presence of photosensitizers has been shown to accelerate telomere shortening *via* DNA damage caused by guanine (G) oxidation,[3–6] based on electron transfer between G and the photoexcited photosensitizers.[7–15] DNA is a principal target of UV-induced cellular damage causing carcinogenesis and photoaging, and it is well known that the most prevalent DNA lesion is the *cis–syn* cyclobutane thymine dimer induced by UVB irradiation.[16–18] However, it is generally accepted that UV-irradiation does not induce pyrimidine dimer formation in telomeric DNA. Recently, Chinnapen and Sen reported a deoxyribozyme consisting of a G-quadruplex structure which is capable of catalyzing a photochemical reaction.[19] It was suggested that UVB-irradiation of the deoxyribozyme leads to the selective excitation of G in the G-quadruplex structure triggering the electron donation to the substrate, that is, the oxidation of G. According to this result, we hypothesized that UVB-irradiation may cause a selective excitation of G followed by its oxidative decomposition in the telomeric structure. In this study, we demonstrate that G shows a higher susceptibility to UVB induced oxidation in the quadruplex DNA than in the normal B-form DNA, suggesting that G oxidation in telomeric DNA may be the key reaction in the shortening of the telomeric DNA caused by UVB irradiation.

The absorption spectra of the human telomeric sequence d[AGGG(TTAGGG)$_3$] in the quadruplex and in the B-form duplex (in the presence of complementary strand d[(CCCTAA)$_3$CCCT]) are shown in Fig. 1. In comparison with the B-form DNA, the quadruplex absorption incorporates a modestly enhanced "tail" in the UVB region which originates from the stacked G-quartet structure,[20] suggesting that selective excitation of G by UVB-irradiation occurs in the quadruplex DNA. First, to assess the feasibility of the selective excitation of G in the telomeric structure, laser flash photolysis of the quadruplex DNA and B-form DNA was performed.

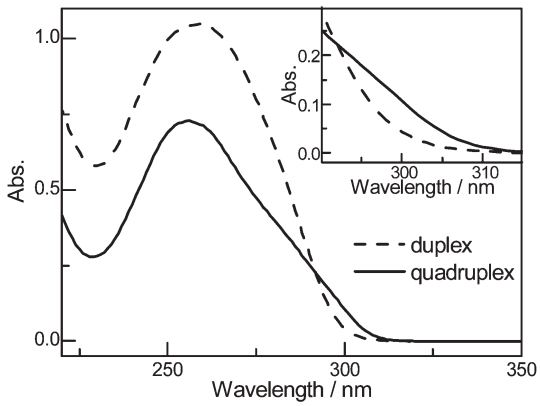

Fig. 1 Ground-state absorption spectra of d[AGGG(TTAGGG)$_3$] in the quadruplex form and in the B-form in the presence of d[(CCCTAA)$_3$CCCT]. Absorption spectra were measured in aqueous solution containing 10 mM Na phosphate buffer (pH 7.0), 100 mM NaCl, and 4.5 μM DNA (strand conc.).

Interestingly, photoirradiation of the quadruplex DNA with a 308-nm laser pulse leads to the formation of an absorption at 630 nm immediately after the flash, which decayed in about 1 μs (Fig. 2). Since its decay rate was accelerated under N_2O saturated conditions this transient absorption is assigned to a solvated electron (e_{aq}), demonstrating the electron ejection from G to the solvent water during the excitation. On the other hand, no transient absorption was observed for the B-form DNA. Recently,

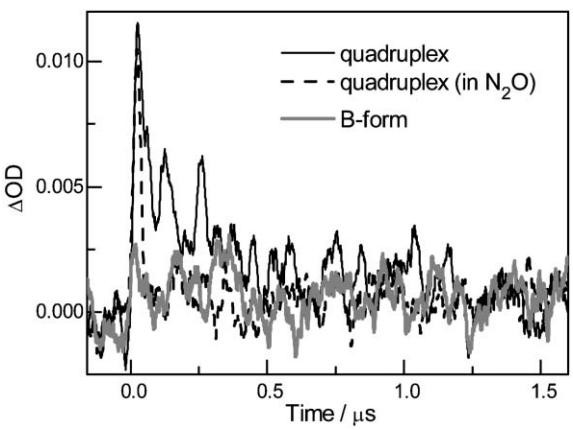

Fig. 2 Decay profiles of the transient absorption measured at 630 nm after the pulsed excitation (308 nm, 15 mJ pulse^{-1}) for the quadruplex DNA d[AGGG(TTAGGG)$_3$] in Ar (black) and in N_2O (dashed line), and for the B-form DNA d[AGGG(TTAGGG)$_3$]/d[(CCCTAA)$_3$CCCT] in Ar (gray). Sample solution contained 100 μM DNA (strand conc.), 100 mM NaCl, and 10 mM Na phosphate buffer (pH 7.0).

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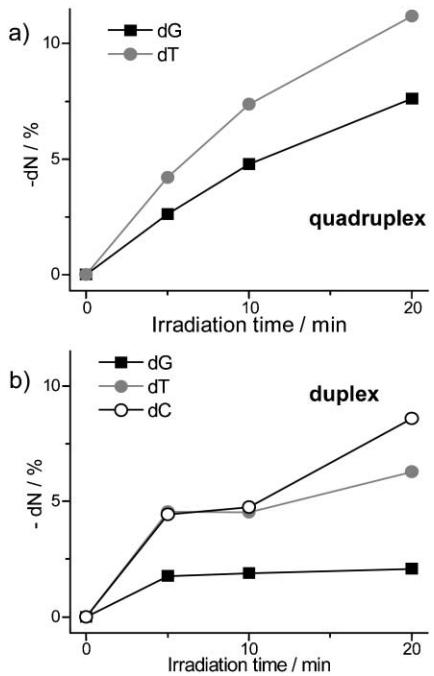

Fig. 3 Photo-degradation of quadruplex and B-form DNA. Each of reaction mixtures containing 10 mM Na phosphate buffer (pH 7.0), 100 mM NaCl, and 40 μM DNA (strand conc.) was photo-irradiated with a high-pressure Hg lamp fitted with a 300-nm cutoff filter. The reaction mixture was directly subjected to enzymatic digestion with snake venom phosphodiesterase, P1 nuclease and alkaline phosphatase and the consumption of nucleosides (−d*N*/%) was quantified by reverse phase HPLC.

we have demonstrated that the electron transfer between G and Ap in the quadruplex DNA occurs less efficiently than in the B-form DNA due to the disruption of the π-overlapping between bases in the telomeric structure.[21] Hence, occurrence of the one-electron oxidation of G in the quadruplex DNA can be explained by the selective excitation of G, and less efficient energy transfer from the excited G to C and T in the telomeric structure than in B-form DNA.

To actually show that G is more easily oxidized by UVB-irradiation in the telomeric DNA than in the B-form DNA, the consumption of G for the quadruplex DNA was compared with that for the B-form DNA during the photoirradiation with a high-pressure Hg lamp fitted with a 300-nm cutoff filter. Interestingly, a high G-consumption was observed in the quadruplex DNA (Fig. 3a). On the other hand, damaged pyrimidine bases dominated in the B-form DNA (Fig. 3b), which is consistent with the fact that the pyrimidine dimer is the major DNA damage caused by the UVB-irradiation. These results clearly show that UVB-irradiation triggers the selective G oxidation in the telomeric DNA.

In conclusion, laser flash photolysis and DNA damage quantification by HPLC of the quadruplex DNA and B-form DNA were performed, and it was clearly demonstrated that UVB-irradiation leads to the selective excitation of G and its subsequent oxidative decomposition in the quadruplex DNA. These results strongly suggested that UVB-irradiation may cause the shortening of the telomeric DNA based on the G oxidative damage.

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