Photochemistry

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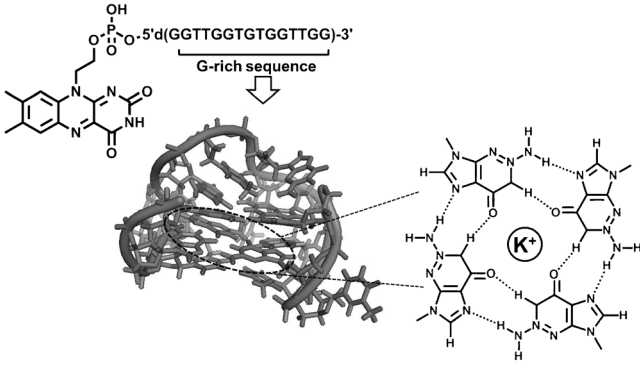
Hole Trapping of G-Quartets in a G-Quadruplex**

Jungkweon Choi, Jongjin Park, Atsushi Tanaka, Man Jae Park, Yoon Jung Jang, Mamoru Fujitsuka, Seog K. Kim, and Tetsuro Majima**

Since reduction and oxidation of DNA are essential processes occurring in various biological phenomena, electron and hole transfer in DNA has drawn recent attention because of its importance and potential application in biological science and nano-biotechnology, respectively. In practice, it is known that the reduction of DNA closely relates to the repair of damaged DNA such as a T-T cyclobutane lesion,[1] whereas the oxidation of DNA promotes oxidative damage, apoptosis, and cancer.[2] Thus, it is important to understand the mechanisms and dynamics of DNA-mediated charge-transfer processes. Excess electron transfer (EET) in DNA has been studied by various techniques, such as laser flash photolysis,[3] γ-ray radiolysis,[4] or product analysis which were used to analyze the cleavage of 5-bromo-2′-deoxyuridine (BrdU) or the T-T dimer in DNA by photoinduced electron transfer.[1,5] The laser flash photolysis is conducted principally on short-length DNA, which is containing four A-T base pairs between two chromophores. Meanwhile, the DNA-mediated hole transfer occurs over a distance greater than 20 nm[6] and the migration of the hole along the DNA involves many steps of short-distance charge-transfer processes between stacked guanine (G) bases because guanine among the four natural DNA bases is most sensitive to oxidation.[7] The hole transfer rate depends on the inserted nucleobase between the G-C base pairs.[3a] In addition, the delocalization of the charge over the stacked G bases along the DNA stem has also been reported.[8]

Most studies on DNA-mediated charge-transfer processes have been performed on a DNA duplex with Watson–Crick base pairing. However, recently, non-B DNAs including G-quadruplexes have attracted great attention as fascinating materials for nanotechnology because of their unique structures. Especially, G-quadruplexes formed from various G-rich sequences have received great attention in biological research because in vitro they block the binding of telomerase and act as a transcriptional repressor element and enzyme to provide cancerous cells immortality.[9] Furthermore, G-quadruplexes are an emerging topic for developing DNA-based molecular electronic devices because of their ability as electron carrier, their unique hole-trapping property,[10] and their high conductance.[11] With hole transfer in DNA, recently, hole trapping has also been regarded as an important process to determine the overall efficiency of hole migration in DNA. Here, we have investigated the hole transfer and trapping in a riboflavin-labeled G-quadruplex using femtosecond (fs) laser flash photolysis and pulse radiolysis. In contrast to duplex DNAs, in which π-π stacking has been believed to be a medium for hole transfer, G-quadruplexes have G-quartet structures composed of four G-bases located in the same plane and have also the stacked G-bases (Scheme 1). Thus, it is expected that the oxidation potential of the G-quartet may be lower than that of the stacking of two or more G, and consequently the G-quadruplex can act as an excellent hole captor. In this study, we found that the charge separation in the G-quadruplex takes place efficiently between riboflavin and the G-bases because of the excellent hole-trapping ability of planar G-quartets. To the best of our knowledge, this is the first report for direct observation of hole injection and trapping in a G-quadruplex structure using laser flash photolysis and pulse radiolysis.



Scheme 1. Chemical structure of riboflavin-labeled oligonucleotide (Rf-G-q) and G-quadruplex (PDB id: 1C35) and G-quartet composed of four guanine bases.

[*] Dr. J. Choi, A. Tanaka, Dr. M. J. Park, Dr. M. Fujitsuka, Prof. Dr. S. K. Kim, Prof. Dr. T. Majima
The Institute of Scientific and Industrial Research (SANKEN)
Osaka University, Mihogaoka 8-1, Ibaraki, Osaka 567-0047 (Japan)
E-mail: majima@sanken.osaka-u.ac.jp
J. Park, Dr. Y. J. Jang, Prof. Dr. S. K. Kim
Department of Chemistry, Yeungnam University
Gyeongsan City, Gyeong-buk, 712-749 (Republic of Korea)
E-mail: seogkim@yu.ac.kr

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To explore the hole injection and trapping in G-quadruplex, we synthesized the riboflavin-labeled oligomer, riboflavin-5′-GGTTGGTGTGGTTGG-3′ (Rf-G-q, Scheme S1 in the Supporting Information). In this study, riboflavin, which is an important endogeneous cellular photosensitizer in vivo and in vitro, is used as a hole injector to DNA bases. The riboflavin derivative (Rf) is attached to the end of the oligomer as shown in Scheme 1 using the method of Kino et al.[12] (see Scheme S1). The G-quadruplex-forming sequence, 5′-GGTTGGTGTGGTTGG-3′ (G-q), is known as a thrombin-binding aptamer, which folds into a monomolecular antiparallel G-quadruplex in the presence of K^+ ions. The formation of a G-quadruplex with the synthesized oligonucleotide in the presence of 100 mM K^+ ions was ensured by its characteristic circular dichroism (CD) spectrum, displaying positive bands at 246 and 295 nm, and a negative band at 266 nm (Figure S1a),[13] indicating that the G-quadruplex has an antiparallel structure. First, we measured the melting temperature (T_m) to elucidate whether the G-quadruplex structure is affected by the covalent attachment of riboflavin. As shown in Figure S1b, the T_m of Rf-G-q in 10 mM potassium phosphate buffer (pH 7.4) containing 100 mM K^+ ions is determined to be 51.2 °C. This value is consistent with that reported by Zaitseva et al.,[14] suggesting that the formation and structure of the G-quadruplex is unaffected by the attachment of riboflavin, and that there is a weak interaction between riboflavin and G-bases in the G-quadruplex and single-stranded sequence at the ground state.

On the other hand, the fluorescence intensities of Rfs attached to single-stranded and G-quadruplex structures are significantly quenched compared with that of free Rf as depicted in Figure 1. Moreover, upon formation of a G-quadruplex, a further decrease in the fluorescence intensity of Rf was observed (Figure 1 and Figure S2), suggesting that Rf in the G-quadruplex interacts more efficiently with the G-bases than in the single-stranded structure. Generally, it is known that the efficient fluorescence quenching of Rf is due to the hole injection to hole acceptors, that is, purines such as adenine and guanine,[15] leading to the formation of a charge-transfer state. Thus, the fluorescence quenching of Rf upon formation of a G-quadruplex results probably from the formation of the charge-transfer state because of the hole transfer from Rf in the singlet excited state (^{1}Rf*) to G-bases.

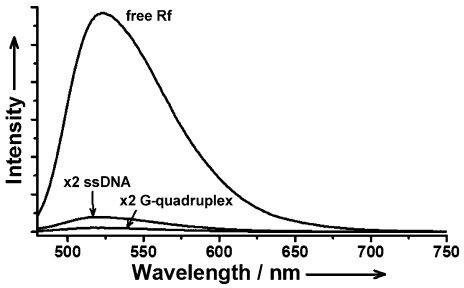


Figure 1. Fluorescence spectra of free Rf, single-stranded Rf-G-q and quadruplex Rf-G-q in 10 mM Tris-HCl buffer (pH 7.4) at room temperature (λ_{ex} = 450 nm).

In other words, this indicates that the oxidation potential of ^{1}Rf* is higher than that of the G-base, which exhibits the lowest oxidation potential among the four DNA bases, consequently resulting in a riboflavin radical anion and a G radical cation.

To confirm the charge transfer between Rf and the G-quartet in the G-quadruplex, we measured the femtosecond-transient absorption spectra for free Rf, single-stranded, and G-quadruplex Rf-G-q in 10 mM Tris-HCl buffer (pH 7.4). The transient absorption spectra of free Rf obtained after laser excitation at 485 nm consist of a positive signal because of the formation and decay of ^{1}Rf* at 480–530 nm and at wavelengths longer than 640 nm, and a negative signal in the 530–640 nm range corresponding to the stimulated emission of free Rf. The decay time of ^{1}Rf* is determined to be 413 ps. In contrast to free Rf, in the early delay times, the single-stranded Rf-G-q shows a strong positive signal (in the ranges of 480–560 nm) with a weak and long-tailed signal located at wavelengths longer than 560 nm. The positive signal of around 508 nm, which is slightly red-shifted compared to that of ^{1}Rf*, quickly decays and changes to a broad and weak positive signal (wavelengths longer than 470 nm; see the middle panel in Figure 2a). The decay profiles of single-stranded Rf-G-q monitored at 510, 570, and 650 nm were expressed by a tri-exponential function with relaxation times of 2 ± 0.2 ps, 40.2 ± 6.6 ps, and > 2 ns (constant). Although the S_1 spectrum of the single-stranded Rf-G-q is slightly red-shifted and the bleaching signal due to the stimulated emission of riboflavin is significantly reduced, the shape of the spectrum observed from single-stranded Rf-G-q is similar to that of free syn-riboflavin (see Figure S3). Thus, the transient signal of single-stranded Rf-G-q observed at about 508 nm certainly originates from the formation and decay of a ^{1}Rf*-labeled oligonucleotide. The reduction of the S_1 state lifetime is mainly due to the charge-transfer process between Rf and G bases. However, we could not observe the formation of the riboflavin radical anion as shown in Figure 2a. This means that the charge recombination is faster or almost identical to the charge transfer (or hole transfer) rate as reported by the Carell group.[16] Thus, the riboflavin radical anion generated by charge transfer is not sufficiently accumulated, resulting in the absence of the absorption band due to the riboflavin radical anion. In addition, interestingly, the transient absorption spectra of single-stranded Rf-G-q observed for longer delay times (purple lines) coincide with that of riboflavin in the triplet excited state (^{3}Rf*),[17] suggesting that ^{1}Rf* is deactivated to ^{3}Rf* through intersystem crossing. This intersystem crossing will be further discussed later.

In contrast to single-stranded Rf-G-q, G-quadruplex Rf-G-q shows distinctly different transient absorption spectra compared with those of free Rf and single-stranded Rf-G-q. As shown in Figure 2a, for early delay times, G-quadruplex Rf-G-q shows a positive signal over a wide wavelength range (see the bottom panel in Figure 2a). The decay profiles of single-stranded Rf-G-q were expressed by a tri-exponential function with relaxation times of 3.6 ± 0.2 ps, 676 ± 205 ps, and > 2 ns (constant; Figure 2b). The absorption maxima around 520 nm are located at longer wavelength than those of

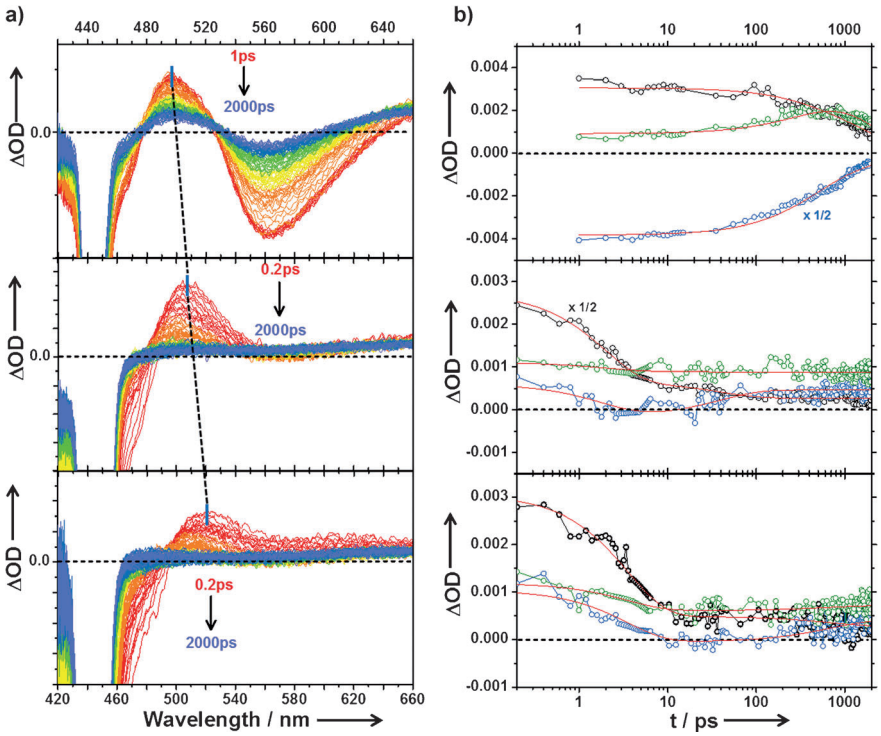


Figure 2. a) Transient absorption spectra of free Rf (top), single-stranded Rf-G-q (middle) and quadruplex Rf-G-q (bottom) after 450 nm laser excitation in 10 mM Tris-HCl buffer (pH 7.4) at room temperature. b) Decay profiles of free Rf (top), single-stranded Rf-G-q (middle) and quadruplex Rf-G-q (bottom) monitored at 510 (black), 570 (blue) and 650 nm (green). Theoretical fits obtained from the global fitting analysis are shown in red color.

free Rf and single-stranded Rf-G-q, implying that the positive signal of 520 nm of G-quadruplex Rf-G-q is not attributed to ^{1}Rf*. This absorption band observed from G-quadruplex Rf-G-q is similar to that of the riboflavin radical anion reported by the Carell group.[16] In addition, the transient absorption spectra observed from G-quadruplex Rf-G-q coincides with the spectrum of the riboflavin radical anion with maximum absorption at 520 nm as reported by Lu et al.[18] This suggests that the positive signal at 520 nm of G-quadruplex Rf-G-q is attributed to the riboflavin radical anion generated by hole injection from Rf to the G-bases, indicating that the G-quadruplex structure induces the efficient charge separation between Rf and G-bases. The efficient charge separation in the G-quadruplex is probably due to the excellent hole trapping ability of planar G-quartets, which is composed of four G-bases bound through a cyclic arrangement of eight Hoogsteen hydrogen bonds. Furthermore, like single-stranded Rf-G-q, the absorption spectra of G-quadruplex Rf-G-q observed for longer delay times are identical with the spectra of ^{3}Rf*,[17] suggesting that the charge-separated state (^{1}Rf*$_{CS}$) undergoes charge recombination to produce ^{3}Rf* in a part.

Here, we consider the hole-trapping ability of planar G-quartets in the G-quadruplex structure. Unfortunately, we could not observe the signal of the G radical cation ($G^{+\bullet}$) generated by charge separation between Rf and G-bases because of a very low extinction coefficient of $G^{+\bullet}$. To elucidate the hole trapping ability of planar G-quartet in G-quadruplex, we measured the transient absorption spectra of G-quadruplex formed from the human telomere sequence, 5′-TAGGG-(TTAGGG)$_3$-TT-3′, using pulse radiolysis (Supporting Information). It is well-known that the human telomere sequence forms a major antiparallel/parallel hybrid (3+1) in the presence of K^+ ions. Pulse radiolysis can induce the generation of $G^{+\bullet}$ by one-electron oxidation. The hydrated electron (e_{aq}^-) generated by pulse radiolysis in aqueous solution containing potassium persulfate ($K_2S_2O_8$) and *tert*-butyl alcohol (for scavenging OH radicals) quickly reacts with a peroxydisulfate ($S_2O_8^{2-}$) to produce a sulfate radical anion ($SO_4^{-\bullet}$), which is a strong oxidant. Then guanine (G) is oxidized to $G^{+\bullet}$ by the sulfate radical anion ($SO_4^{-\bullet}$; see the Supporting Information). Figure 3a shows the transient absorption spectra obtained by pulse radiolysis of G-quadruplex in 20 mM potassium phosphate buffer containing 20 mM $K_2S_2O_8$ and 0.1M *tert*-butyl alcohol at early delay times (Δt = 50–1000 ns). The absorption spectra observed at Δt = 50 ns are characterized by a broad signal with absorption maxima at 460 nm (Figure 3a). This signal coincides with the spectrum of $G^{+\bullet}$ reported by Kobayashi et al.,[19] indicating that the absorption spectra observed at Δt = 50 ns is attributed to $G^{+\bullet}$and $G^{+\bullet}$ is efficiently formed even in G-quadruplex. It has been reported that the stacking of two or more G bases induces a lower ionization potential (IP) compared with that of a single isolated G, making stacked G sites thermodynamically more favorable for hole trapping.[20] Indeed, Kobayashi et al. reported that the stabilization of $G^{+\bullet}$ due to the stacking interaction of two or three consecutive G bases in duplex DNA leads to a spectral shift in the absorption band of $G^{+\bullet}$.[19b] In this study, we found that the spectrum of $G^{+\bullet}$ observed from the G-quadruplex is relatively red-shifted compared to those spectra of two or three consecutive G bases, supporting that the hole trapping in the planar G-quartet of the G-quadruplex is favored because of the delocalized positive charge along the more extended π obitals. Meanwhile, the spectra with absorption maxima at 390 and 550 nm observed at longer delay times are due to the neutral radical of G [$G^{+\bullet}$(-H)], which is the deprotonated species of $G^{+\bullet}$. The rate constant for the formation of $G^{+\bullet}$(-H) is determined to be $4.0 \pm 0.2 \times 10^6$ s^{-1} that is slightly smaller than those constants reported in the literature.[19b,21] This means that it is more difficult to observe the deprotonation of $G^{+\bullet}$ in a G-quadruplex than in a duplex DNA structure.

As mentioned above, it is note-worthy that the transient absorption spectra of single-stranded and G-quadruplex Rf-G-q observed at longer delay times coincide with the

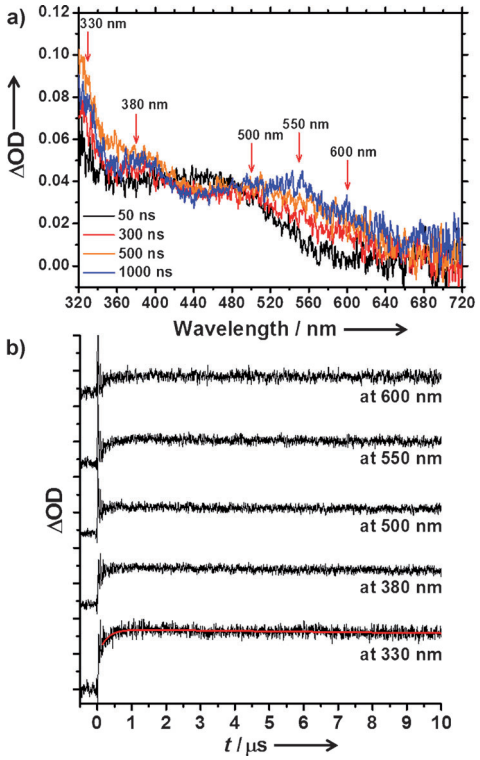
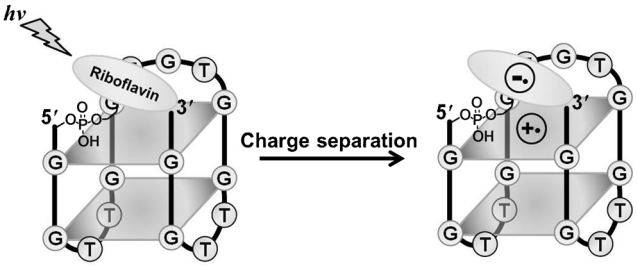


Figure 3. a) Transient absorption spectra obtained by pulse radiolysis of the G-quadruplex formed from the 5′-TAGGG(TTAGGG)$_3$TT-3′ sequence in 20 mM potassium phosphate buffer (pH 7.4). b) Decay profiles of the G-quadruplex formed from the 5′-TAGGG(TTAGGG)$_3$TT-3′ sequence in 10 mM potassium phosphate buffer (pH 7.4) monitored at 330, 380, 500, 550, and 600 nm (from bottom to top), respectively. Theoretical fitted curves obtained from the global fitting analysis are shown in red color.

sepctrum of ^{3}Rf*,[17] indicating when Rf is labeled to an oligonucleotide, ^{1}Rf* or ^{1}Rf*$_{CS}$ is quickly deactivated through intersystem crossing or charge recombination to produce ^{3}Rf*, respectively. This fast intersystem crossing was observed for free FMN (flavin mononucleotide) by Li and Glusac.[17] They reported that in case of free FMN, the process of intersystem crossing from FMN in the singlet excited state to FMN in the triplet excited state occurs with a rate constant of 2×10^8 s^{-1}. Considering the longer decay component and the rise component observed with 570 and 650 nm (Figure 2b), respectively, we analyzed that the process of the intersystem crossing for single-stranded and G-quadruplex Rf-G-q occurs at rate constants of 2.5×10^{10} and 1.5×10^9 s^{-1}, respectively. Although the intersystem crossing for riboflavin-labeled oligonucleotide occurs more quickly than for the free flavin compound, the transition from ^{1}Rf*$_{CS}$ to ^{3}Rf* is relatively slow relative to that from ^{1}Rf* to ^{3}Rf*. This is probably due to the stabilization of ^{1}Rf*$_{CS}$ by the hole trapping in the planar G-quartet of the G-quadruplex.

In conclusion, we have investigated the hole transfer and trapping in a G-quadruplex using laser flash photolysis and pulse radiolysis. As a result, we found that the G-quartet of the G-quadruplex have lower oxidation potentials compared with that of a single isolated G and two or three consecutive G bases. Indeed, the results presented here reveal that the



Scheme 2. Hole transfer and trapping by G-q in Rf-G-q.

charge separation in the G-quadruplex Rf-G-q takes place efficiently between riboflavin and G-bases due to the excellent hole-trapping ability of planar G-quartets (Scheme 2). This means that G-quadruplexes can serve as traps of oxidative damage in the genome. In addition, this efficient charge transfer between riboflavin and G-bases resulted in a relatively slow transition from ^{1}Rf*$_{CS}$ to ^{3}Rf*. We believe this will certainly contribute to the development of molecular electronic devices incorporating a G-quadruplex and help to understand the mechanisms on the damage and repair of DNA in terms of biomedical science.

Experimental Section

Full experimental details and characterization of compounds can be found in the Supporting Information. Rf-G-q and G-rich sequence (5′-TAGGG-(TTAGGG)$_3$-TT-3′) were synthesized on an Applied Biosystems 3400 DNA synthesizer with standard solid-phase techniques and purified on a JASCO HPLC with a reversed phase C-18 column with an acetonitrile/50 mM ammonium formate gradient. The DNAs were characterized by digestion with nuclease P1 and alkaline phosphatase and by MALDI-TOF mass spectra (Figure S4).

The steady-state fluorescence and CD spectra were measured using Horiba FluoroMax-4 and JASCO CD-J720, respectively.

The femtosecond transient absorption spectra were measured by the pump–probe method using a regenerative amplified titanium sapphire laser (Spectra Physics, Spitfire Pro F, 1 kHz) pumped by a Nd: YLF laser (Spectra Physics, Empower 15). Pulse radiolysis experiments were performed using an electron pulse (27 MeV, 11 A, 8 ns, 0.8 kGy per pulse) from a linear accelerator at Osaka University. Full experimental details can be found in the Supporting Information.

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