J|A|C|S COMMUNICATIONS

Published on Web 04/23/2009

"Signal-On" Detection of DNA Hole Transfer at the Single Molecule Level

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The π-stacked array of nucleic acid base pairs in a DNA duplex mediates charge transfer over long distances.[1,2] DNA is a highly organized scaffold and an effective medium for long-distance charge transfer. Hence, it can be utilized to develop nanoelectronics and sensing devices.[3−6]

Long-distance charge transfer through DNA has been studied mainly through biochemical assays,[7] time-resolved spectroscopic measurements,[8,9] and electrochemical analysis.[10] The fluorescence detection technique is intrinsically sensitive[11] and is applicable to single-molecule spectroscopy.[12] However, this technique has not been satisfactorily used to study hole transfer through DNA (DNA HT). Single-molecule spectroscopy is a valuable method for understanding the biological significance of DNA HT occurring within a cell where DNA is mainly bound to various kinds of proteins. In this context, the single-molecule assay based on fluorescence detection offers an alternative and valuable approach for a better understanding of DNA HT chemistry. In our previous study, we have described a method for the single-molecule detection of DNA HT from fluorescence signal changes after remote oxidation of the fluorophore through DNA HT.[13] In this system, the spontaneous photobleaching of the fluorophore during single-molecule observation is unavoidable, and it may lead to observational errors. Therefore, further improvement of this system is required. In this communication, we report the single-molecule detection of DNA HT based on fluorescence signal generation.

The design of the single-molecule detection system of DNA HT based on fluorescence signal generation is shown in Figure 1a. DNA is conjugated with fluorescence resonance energy transfer (FRET) pairs, Black Hole Quencher (BHQ),[14] and tetramethyl-6-carboxyrhodamine (TAMRA). BHQ and TAMRA are used as a fluorescence quencher and fluorophore, respectively (Table 1). A photosensitizer (naphthalimide, NI) is attached on the opposite side of DNA. In this assembly, the fluorescence of TAMRA is completely quenched by the BHQ via FRET (off-state).[15] The photoexcitation of NI results in a long-lived positive charge (hole) in the DNA through adenine(A)-hopping (Figure 1b).[16,17] The hole freely migrates through the DNA base stack by between the guanine bases (Figure 1c).[18] Once a hole is trapped at the site of BHQ, BHQ is oxidized and decomposed. This leads to the cancelation of FRET. The fluorescence signal can be recovered from TAMRA in this assembly through remote oxidation of BHQ by DNA HT (on-state). As a result, the fluorescent state switches from off-state to on-state. This process transduces the DNA HT event into the fluorescence signal.

The oxidation of BHQ and the fluorescence recovery of TAMRA were first characterized in solution experiments. The oxidative degradation of BHQ molecules by DNA HT was investigated by UV−visible absorption measurements. Strong and broad ground-state absorption of BHQ around 600 nm for NI/Q decreased with increasing irradiation time (Figure 2a), indicating that BHQ was oxidized and decomposed by DNA HT. This irreversible oxidation and selective decomposition of BHQ by DNA HT was also confirmed from HPLC analysis (see SI). These results clearly suggest that the hole generated

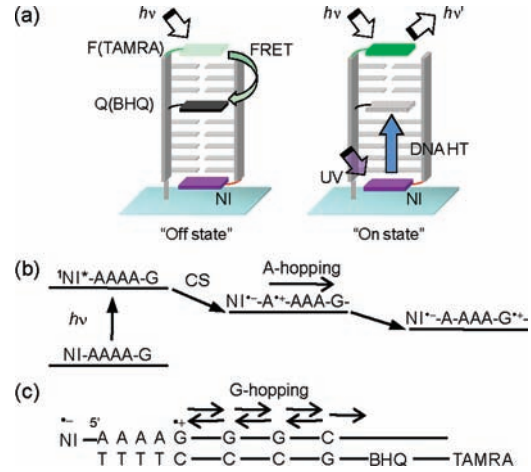

Figure 1. (a) Schematic illustration of single-molecule detection of DNA HT by fluorescence signal generation. (b) Mechanism of charge separation through A-hopping after the excitation of NI with UV light. (c) Hole transfer process through G-hopping. The quencher, BHQ, is oxidized and decomposed through DNA HT.

Table 1. DNA Sequences Used in This Study[a]

DNA	Sequence
NI	5′-NI-AAAAGAGCT GACAAAAAAAA-3′
Q	3′-TTTTCTCGACTG-(T-BHQ)-TTTTTTT-5′
FQ	3′-TTTTCTCGACTG-(T-BHQ)-TTTTTTT-TMR-5′
M1	5′-NI-A**G**AAGAGCTGACAAAAAAAA-3′
M2	5′-NI-AAAAA**A**AGCTGACAAAAAAAA-3′
BT-FQ	3′-BT-TTTTT-TTTTCTCGACTG-(T-BHQ)-T_7-TMR-5′

[a] BHQ-attached thymine and TAMRA are denoted by T-BHQ and TMR, respectively. Mismatch bases are bold in the sequence.

in the DNA by UV irradiation is selectively trapped at BHQ rather than at the nucleobases, particularly G bases. Fluorescence recovery through oxidation of BHQ by DNA HT was monitored by steady-state fluorescence measurements (Figure 2b). Before UV irradiation, very weak fluorescence was observed. This indicates that fluorescence quenching of TAMRA was achieved by BHQ through FRET. Upon irradiation, a significant increase in the fluorescence emission was observed. The recovery factor (I/I_0), which is defined as the ratio of fluorescence intensity before (I_0) UV irradiation to the fluorescence intensity after (I) UV irradiation at 580 nm, was calculated to be ~8 for NI/FQ. This increase in fluorescence emission is sufficient to distinguish between the fluorescence off-state and on-state in single-molecule experiments (vide infra). To confirm that the fluorescence recovery was due to DNA HT, two kinds of DNA samples were investigated (Figure 2b, inset). One was a DNA solution containing DNA without NI, and the other containing a mixture of DNA without NI and DNA without TAMRA. There was no increase in fluorescence emission for both solutions. In other words, the decomposition of BHQ did not take place through direct excitation and intermolecular

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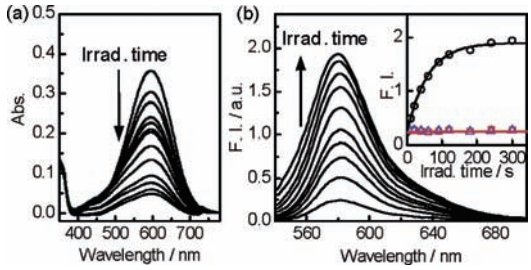

Figure 2. (a) Absorption spectral changes of BHQ after irradiation (0−120 s) at 365 nm for NI/Q. Sample solutions contained 10 μM DNA in 20 mM Na phosphate buffer (pH 7.0) and 100 mM NaCl. (b) Fluorescence spectral recovery after UV irradiation for NI-FQ. (Inset) Fluorescence intensity at the emission peak of TAMRA as a function of irradiation time for NI-FQ (black circles), DNA lacking NI (red squares), and the mixture of DNA lacking NI and DNA lacking TAMRA (blue triangles).

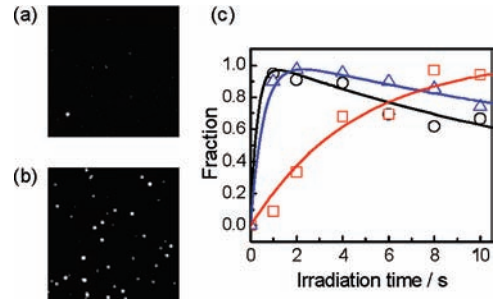

Figure 3. Single-molecule fluorescence images (a) before and (b) after UV irradiation at 365 nm. (c) Plots of the increase number of bright spots as a function of irradiation time for NI/BT-FQ (black circles), M1/BT-FQ (red squares), and M2/BT-FQ (blue triangles). Relative recovery fractions were calculated by normalizing at the maximum number for each sample.

oxidation. These results clearly indicate that the oxidative decomposition and fluorescence recovery were induced by DNA HT.

Fluorescence signal generation by DNA HT in the designed DNA assembly was applied to single-molecule fluorescence measurements. Biotinylated DNA possessing TAMRA and BHQ (BT-FQ) was immobilized on glass surfaces through biotin−streptavidin interaction. Subsequently, NI-modified cDNA (NI, M1, and M2) was hybridized to surface-immobilized DNA to form double strand DNA. After hybridization, the modified glass surfaces were washed with buffer solutions and imaged by total-internal reflection fluorescence microscopy. Before UV irradiation, almost no bright spots were observed as expected from the ensemble solution experiments. This indicates that the fluorescence emission from TAMRA is effectively quenched by the neighboring BHQ (Figure 3a). After UV irradiation, many bright spots were observed (Figure 3b), which clearly indicate that the fluorescence signal generation was triggered by DNA HT and the DNA HT event was specifically detected at the single molecule level. The time course of the number of bright spots against the irradiation time is shown in Figure 3c. The number of bright spots depends on the irradiation time. Prolonged UV irradiation caused the photobleaching of TAMRA, which was attributed to the oxidation of TAMRA by DNA HT after BHQ decomposition. These results show that the fluorescence signal from TAMRA recovered after oxidative decomposition of BHQ by DNA HT was quenched by oxidation of TAMRA through the next DNA HT.

The effects of single-base mismatch on fluorescence recovery by DNA HT were also investigated by single molecule spectroscopy (Figure 3c). A significant suppression in the fluorescence signal by DNA HT was observed when an A−C mismatch was incorporated near NI (M1/BT-FQ). This indicates that the mismatch base pair in DNA prevents DNA HT because of the disruption in the integrity of the π-stacked order of the double helical DNA. However, the mismatch base pair in M2/BT-FQ showed a weak suppression of fluorescence recovery. This result can be explained by the rapid charge transfer in the A-tract sequence.[19,20] In the M2/BT-FQ assembly, the A-tract sequence between NI and G caused efficient charge separation and oxidation of BHQ.

In conclusion, we demonstrated a method to study DNA HT using fluorescence signal detection. We have established the single-molecule detection of DNA HT at the single molecule level based on the fluorescence generation by combining the oxidative reaction of the quencher molecule through DNA HT and the cancelation of FRET. This "signal-on" detection system makes it possible to detect DNA HT in the individual DNA and base-pair mismatch in the target DNA with high sensitivity and accuracy.[20] The single-molecule detection technique will be used to investigate DNA HT in a DNA−protein complex to further understand the biological role of DNA HT in the living cell.[21]

Acknowledgment. This work has been partly supported by a Grant-in-Aid for Scientific Research (Project 17105005, 19350069, Priority Research (477), and others) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of the Japanese Government.

Supporting Information Available: Experimental procedures for the synthesis and spectroscopic analysis, and HPLC analysis of the reaction. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA9009919