Bioorganic & Medicinal Chemistry Letters 20 (2010) 994–996

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Fluorescent analysis of excess electron transfer through DNA

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ARTICLE INFO

Article history:
Received 24 November 2009
Revised 10 December 2009
Accepted 11 December 2009
Available online 21 December 2009

Keywords:
DNA
Excess electron transfer
Fluorescent probes
Disulfides

ABSTRACT

The DNA base stack provides unique features for the efficient long-range charge transfer. For the purpose of investigating excess electron transfer process through DNA, we developed a new method for fluorescence analysis of excess electron transfer based on reductive cleavage of a disulfide bond and a thiol-specific fluorescent probe. Excess electron transfer was detected by monitoring the fluorescence of emissive pyrene monomer generated by the reaction of pyrene maleimides with the cleaved disulfide bond (thiols). Mechanism of reductive cleavage of disulfides through excess electron transfer and subsequent reaction with the fluorescent probes were discussed. This facile and sensitive detection by fluorescence method can be applied for mechanistic study of excess electron transfer.

Charge transfer in DNA has been extensively studied in the last decade because of its relevance to biological consequences such as DNA damage and DNA repair and its importance in development of DNA-based nano-devices.[1–3] Number of experiments using time-resolved spectroscopic and several other chemical methods have showed that positive charge injected into the bases of DNA can move through DNA π-stacks over significant distances.[3,4] It is now accepted that two mechanisms of superexchange for short transfer distances and hole or polaron hopping for long-range transfer are involved in DNA-mediated positive charge transfer.[2,5]

When compared to the hole migrations through DNA, less attention has been paid for excess electron transfer through the base stacks of DNA.[6] DNA-mediated excess electron transfer has been studied by several research groups.[7–9] A time-resolved spectroscopic study has provided the dynamics of electron transfer processes such as electron injection, charge recombination, and electron transfer occurred in DNA.[10–12] A chemical probing has been a useful mean for analysis of excess electron transfer. Thymine cyclobutane dimer and 5-halouracil have been used as an electron trap that is reductively cleaved by an electron charged into DNA helixes.[9,12] In these studies, the weak distance dependences of the electron transfer between the electron injected bases and the electron traps in DNA have been demonstrated.[7,13]

Although there exists several methods to analyze the excess electron transfer in DNA,[9] an easier and more convenient method is necessary to study electron transfer between a donor and an accepter thorough a DNA-bridge. In this Letter, we describe a new method for analysis of excess electron transfer through DNA. Our method involves the injection of excess electron from a photo-excitable electron donor into DNA, the reductive cleavage of a disulfide bond as an electron accepter or trap that is incorporated into DNA, and the subsequent fluorescent detection of the cleavage product, thiol, with a thiol-specific fluorescent probe.

Thiols and disulfides can behave as redox-responsive groups because switching between oxidized (thiol) and reduced forms (disulfide) are regulated by redox signal.[14] Tanabe et al. demonstrated that reduction of disulfides by solvated electrons generated by X-radiolysis was utilized to induce disulfide exchange reactions and ligation of oligonucleotides.[15] Recent studies have shown that thiol and disulfide respond to the charge transfer through DNA. Disulfide bond formation triggered by remote oxidation through DNA π-stack was characterized by product analysis.[16] Electrochemical reduction of a disulfide bond imbedded in DNA was investigated electrochemically on a graphite electrode, showing reductive cleavage via concerted $2e^-$, $2H^+$ process.[17]

The fluorescence detection of excess electron transfer in DNA is schematically shown in Figure 1. We have designed DNA conjugates possessing a photosensitizer (phenothiazine, PTZ) at the 5′-end and a disulfide bond incorporated internally into DNA. PTZ serves as an electron donor and a disulfide as an electron acceptor, because PTZ in the excited state has high reduction potential ($^*E_{ox} = -2.7$ V) that is enough to reduce an adjacent pyrimidine base.[18] It is anticipated that an electron injected from PTZ into the nearby base in DNA should migrate to the disulfide bond, and subsequent one-electron reduction of the disulfide should produce a disulfide radical anion ($RSSR^{\cdot -}$).[17] This unstable intermediate immediately splits into thiyl radical ($RS^{\cdot}$) and thiyl anion (RS^-).[19]

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doi:10.1016/j.bmcl.2009.12.052

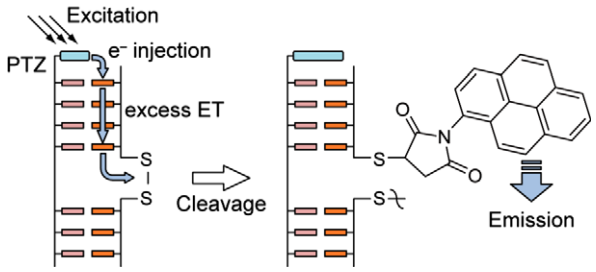

Figure 1. Strategy for fluorescence analysis of excess electron transfer through DNA.

In order to convert the cleavage reaction into a measurable fluorescent signal, we use *N*-(1-pyrene) maleimide (Py-MI) as a thiol-specific fluorescent dye. The thiyl anion is expected to react with the fluorescent probe specifically, resulting in the fluorescence signal to analyze the DNA-mediated electron transfer. It is importantly noted that Py-MI is nonfluorescent until it reacts with thiols and the thiol-adducts of Py-MI show strong fluorescence (>100-fold) compared with unreacted Py-MI.[20] Sequences of DNA conjugates used in this study are shown in Chart 1. The PTZ-disulfide-DNA conjugates (PZnSS) was chemically synthesized by using a PTZ-phosphoramidite and a commercially available disulfide modifier according to the previous report.[21] The disulfide bond was incorporated in PZnSS that are separated by A-T base pairs (n = 0–4) from PTZ at the 5′-terminus. Consecutive sequence of A-T base pairs between PTZ and SS was used because of the efficient excess electron transfer through consecutive T sequence compared with mixed sequence.[22]

Figure 2 shows fluorescence spectra obtained for PZ2SS in the presence of Py-MI. Samples containing PZ2SS and Py-MI were irradiated with UV light (365 nm) using transilluminator and then spectral change were measured by fluorescence spectrometer. Before UV irradiation, no fluorescence of Py-MI was observed, showing that pyrene is in a nonfluorescent state due to the maleimide group.[20] After excitation of PTZ with UV light, emission from pyrene appeared. Fluorescence signal increased as increasing the irradiation time (Fig. 2, inset).[23] In the case of DNA lacking the disulfide, a fluorescence generation was not observed, which means that the fluorescent adducts was produced by the cleavage of the disulfide. Cleavage yields for PZ2SS can be calculated by comparing the fluorescence intensities obtained for PZ2SS and for Py-MI reacted with 2-mercaptoethanol. Yield for PZ2SS after 60 min irradiation was estimated to be 0.8%. This relatively low reaction yield can be attributed to slow cleavage reaction of disulfide radical anion relative to charge recombination and/or slow electron hopping from DNA to the disulfide.[24] In principle, the reductive cleavage of one disulfide can generate two free thiols, meaning the formation of two emissive pyrenes. If it occurred, excimer emission of pyrenes should appear due to the close proximity to each other.[25] However, broad emission assigned to

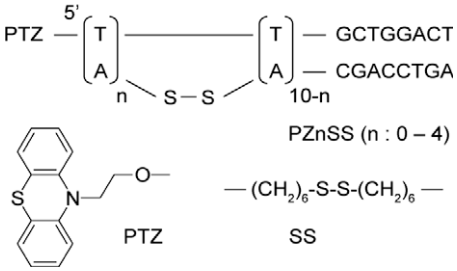

Chart 1. Sequences of DNA used in this study.

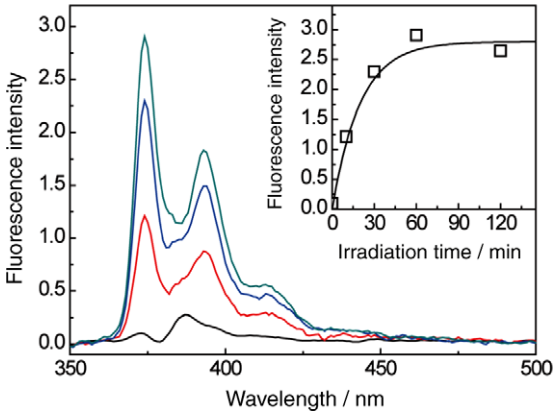

Figure 2. Fluorescence spectral changes for PZ2SS (1 μM) obtained upon excitation at 340 nm after UV irradiation in the presence of Py-MI (100 nM) in 20 mM Na phosphate buffer (pH 7.0) and 100 mM NaCl. Inset: Fluorescence intensity at 375 nm as a function of irradiation time.

excimer formation around 470 nm was not observed. Possible reasons may include dissociation of the short strand that is produced by cleavage of the disulfide or inefficient second one-electron reduction.[17]

Distance dependence of the excess electron transfer was investigated by monitoring the change in the fluorescence intensity for PZnSS (Fig. 3). Distance between PTZ and SS was controlled by changing the number of AT base pairs. When SS was in contact with PTZ (PZ0SS), very low cleavage yield was obtained. This result indicates the direct reduction of disulfide and rapid charge recombination prior to the cleavage of disulfide radical anion. Electron injection and charge recombination process for DNA have been well characterized by using time-resolved transient spectros-

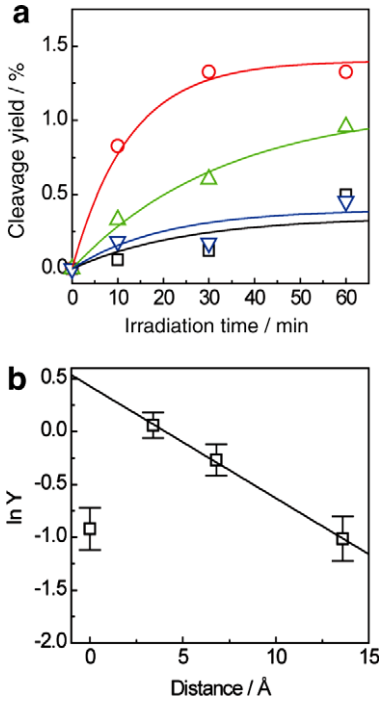

Figure 3. (a) Cleavage yields for PZnSS against irradiation time (n = 0:black squares, n = 1:red circles, n = 2:green up triangles, n = 4:blue down triangles). Fluorescence intensities at 374 nm of 0–0 band of pyrene emission were used for the calculation. (b) Distance dependence of the reaction yields for PZnSS. A straight line was obtained from the fitting without PZ0SS.

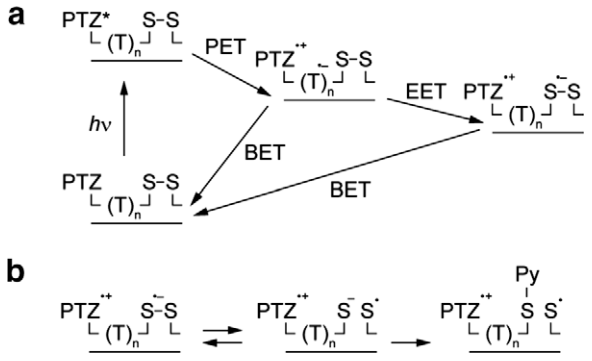

Figure 4. (a) Reduction of disulfide via excess electron transfer and (b) reductive cleavage of disulfide and the following reaction with pyrene maleimide. PET, BET, and EET stand for photoinduced electron transfer, back electron transfer, and excess electron transfer, respectively.

copy.[10,26] The charge recombination in contact pair proceeds in subnanosecond timescale, which is much faster than the reductive cleavage of disulfide and the subsequent reaction with Py-MI.[27,28] Consequently, adduct formation was significantly suppressed. When the disulfide is separate from PTZ by several AT base pairs, the cleavage reaction can compete with the charge recombination, resulting in the increase in the fluorescence signal of pyrene. This is due to the fact that the charge recombination rates decrease exponentially with the distance.[29] Logarithm of the reaction yields (ln *Y*) obtained after 60 min irradiation are plotted against the distance (Δr) between PTZ and SS. Linear relationship was observed, providing a slope of 0.1 Å which corresponds to apparent distance dependence of the excess electron transfer. A shallow distance dependence in their cleavage yields indicates that the disulfide was reduced by a hopping mechanism rather than a direct electron transfer from PTZ in the excited state.[30] Mechanistic scheme for the reduction of disulfide through the excess electron transfer and the following cleavage of the disulfide is shown in Figure 4. Excitation of PTZ by UV irradiation generates the charge separated state between PTZ and adjacent thymine through photoinduced electron transfer (PET). This process corresponds to electron injection. Electron on thymine base migrates via hopping, and is trapped by disulfide (Fig. 4a). Cleavage of disulfide radical anion prior to the charge recombination leads to the formation of pyrene adducts exhibiting fluorescent emission (Fig. 4b).

In conclusion, we have shown the fluorescence methods to detect excess electron transfer by using the reductive cleavage of the disulfides and the thiol-specific fluorescent probes. Excess electron transfer was detected by monitoring the fluorescence of pyrene monomer. This facile and sensitive detection by fluorescence method can be applied for mechanistic study of excess electron transfer.

Acknowledgments

This work was partly supported by a Grant-in-Aid for Young Scientists (Start-up) and the Research Foundation for Opto-Science and Technology.

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