

# Sequence-specific electron injection into DNA from an intermolecular electron donor

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## ABSTRACT

Electron transfer in DNA has been intensively studied to elucidate its biological roles and for applications in bottom-up DNA nanotechnology. Recently, mechanisms of electron transfer to DNA have been investigated; however, most of the systems designed are intramolecular. Here, we synthesized pyrene-conjugated pyrrole-imidazole polyamides (PPIs) to achieve sequence-specific electron injection into DNA in an intermolecular fashion. Electron injection from PPIs into DNA was detected using 5-bromouracil as an electron acceptor. Twelve different 5-bromouracil-containing oligomers were synthesized to examine the electron-injection ability of PPI. Product analysis demonstrated that the electron transfer from PPIs was localized in a range of 8 bp from the binding site of the PPIs. These results demonstrate that PPIs can be a useful tool for sequence-specific electron injection.

## INTRODUCTION

The migration of negative and positive charges in DNA, which are excess electrons and holes, respectively, has attracted considerable interest recently, in the fields of both biology and engineering. For example, in biology, hole transfer in DNA may promote oxidative DNA damage from a remote site, which may define mutation hotspots in the genome (1–3). Electron transfer in DNA is used during the repair of ultraviolet-induced cyclobutane pyrimidine dimers in DNA, in which electron transfer from FADH<sup>-</sup> to the dimer lesion is crucial (4,5). In engineering, electron transfer in DNA is expected to be applied to the development of DNA-based electronic nanodevices (6–9). To investigate the mechanism of charge transfer in DNA,

various model systems consisting of a photosensitizing electron donor and acceptor covalently attached to DNA have been used (10–17). Although these intramolecular systems can efficiently inject electrons into DNA, it is difficult, or sometimes even impossible, to conjugate the electron donor at the desired place, especially in long DNA molecules, because of synthetic difficulties. Sequence-specific electron injection in an intermolecular fashion is necessary for further studies in biology and for more flexible applications in the field of engineering.

Here, we synthesized pyrene-conjugated pyrrole-imidazole polyamides (PPIs) to achieve sequence-specific electron injection from intermolecular electron donors into DNA. In PPIs, the pyrene moiety is used as an electron donor (8,11), and pyrrole-imidazole polyamide, which recognizes each of the four Watson–Crick base pair sequences, is used for sequence-specific binding to DNA (18–21). To detect electron transfer in DNA, 5-bromouracil (5-BrU)-containing DNA was used as an electron acceptor. 5-BrU is readily reduced into its anion radical, which generates the uracil-5-yl radical in DNA and immediately abstracts hydrogen from the deoxyribose backbone or the appropriate hydrogen donor; this leads to the generation of uracil, which can be detected using various methods (10,11,22,23). We demonstrated that, under irradiation conditions, our PPI sequences specifically inject electrons into the 5-BrU residue in DNA.

## MATERIALS AND METHODS

### General

Phosphoramidites were obtained from Proligo or Glen Research. Oligonucleotides were synthesized on an ABI DNA synthesizer (Applied Biosystems). After purification by high performance liquid chromatography (HPLC), synthesized oligonucleotides were checked by HPLC and ESI-TOF-MS (Bruker). <sup>1</sup>H NMR spectra of PPIs were recorded on a JEOL JNM ECA-600 spectrometer

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(600 MHz for  $^1\text{H}$ ), with chemical shifts reported in parts per million relative to residual solvent and coupling constants reported in Hz. The following abbreviations were applied to spin multiplicity: s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet).

### $^1\text{H}$ NMR of PPI 1 and PPI 2

PPI 1:  $^1\text{H}$  NMR (600 MHz, DMSO- $d_6$ )  $\delta$  10.295 (s, 1H; NH), 10.270 (s, 1H; NH), 9.939 (s, 1H; NH), 9.929 (s, 1H; NH), 9.903 (s, 1H; NH), 9.895 (s, 1H; NH), 9.890 (s, 1H; NH), 9.840 (s, 1H; NH), 8.361 (d, 1H; CH,  $J = 8.84$ ), 8.259 (s, 1H; CH), 8.247 (s, 1H; CH), 8.208 (d, 1H; CH,  $J = 2.72$ ), 8.194 (d, 1H; CH,  $J = 4.08$ ), 8.129 (d, 1H; CH,  $J = 8.84$ ), 8.107 (d, 1H; CH,  $J = 8.84$ ), 8.020–8.073 (m, 5H; NH), 7.987 (t, 1H; CH,  $J = 5.78$ ), 7.914 (d, 1H; CH,  $J = 8.16$ ), 7.865 (t, 1H; CH,  $J = 5.78$ ), 7.537 (s, 1H; CH), 7.502 (s, 1H; CH), 7.383 (d, 1H; CH,  $J = 2.04$ ), 7.278 (d, 1H; CH,  $J = 2.04$ ), 7.224 (d, 1H; CH,  $J = 1.36$ ), 7.217 (d, 1H; CH,  $J = 2.04$ ), 7.161 (s, 2H; CH), 7.147 (d, 1H; CH,  $J = 2.04$ ), 7.073 (s, 2H; CH), 6.938 (d, 1H; CH,  $J = 2.04$ ), 6.884 (t, 2H; CH,  $J = 2.04$ ), 3.973 (s, 3H; CH), 3.940 (s, 3H; CH), 3.866 (s, 4H; CH), 3.851 (s, 4H; CH), 3.843 (s, 4H; CH), 3.829 (s, 8H; CH), 3.804 (s, 4H; CH), 3.078–3.155 (m, 6H; CH), 2.988–3.024 (m, 4H; CH), 2.417 (t, 2H; CH,  $J = 7.14$ ), 2.386 (m, 4H; CH,  $J = 3.74$ ), 2.355 (t, 2H; CH,  $J = 7.14$ ), 2.283 (t, 2H; CH,  $J = 7.82$ ), 2.250 (t, 2H; CH,  $J = 7.14$ ), 2.204 (t, 2H; CH,  $J = 7.14$ ), 1.813 (t, 2H; CH,  $J = 7.48$ ), 1.741 (t, 2H; CH,  $J = 7.82$ ), 0.855 (t, 1H; CH,  $J = 7.13$ ). ESI-TOF-Mass:  $m/z$  calcd. C<sub>84</sub>H<sub>96</sub>N<sub>24</sub>O<sub>13</sub> [M+2H]<sup>+</sup> 1650.7589; found 1650.7409.

PPI 2:  $^1\text{H}$  NMR (600 MHz, DMSO- $d_6$ )  $\delta$  10.268 (s, 1H; NH), 10.244 (s, 1H; NH), 10.034 (s, 1H; NH), 9.942 (s, 1H; NH), 9.904 (s, 1H; NH), 9.896 (s, 1H; NH), 9.868 (s, 1H; NH), 9.858 (s, 1H; NH), 8.367 (d, 1H; CH,  $J = 8.84$ ), 8.263 (s, 1H; CH), 8.250 (s, 1H; CH), 8.214 (d, 1H; CH,  $J = 2.04$ ), 8.199 (d, 1H; CH,  $J = 3.40$ ), 8.128 (d, 1H; CH,  $J = 8.84$ ), 8.109 (d, 1H; CH,  $J = 8.84$ ), 7.972–8.058 (m, 5H; NH), 7.927 (s, 1H; CH), 7.914z (s, 1H; CH), 7.864 (t, 1H; CH,  $J = 5.78$ ), 7.537 (s, 1H; CH), 7.494 (s, 1H; CH), 7.327 (d, 1H; CH,  $J = 1.36$ ), 7.266 (d, 1H; CH,  $J = 1.36$ ), 7.257 (d, 1H; CH,  $J = 2.04$ ), 7.233 (d, 1H; CH,  $J = 1.36$ ), 7.181 (d, 1H; CH,  $J = 1.36$ ), 7.171 (d, 1H; CH,  $J = 1.36$ ), 7.135 (d, 1H; CH,  $J = 2.04$ ), 7.087 (d, 1H; CH,  $J = 2.04$ ), 7.060 (d, 1H; CH,  $J = 1.36$ ), 6.918 (d, 1H; CH,  $J = 1.36$ ), 6.879 (d, 1H; CH,  $J = 1.36$ ), 6.848 (d, 1H; CH,  $J = 1.82$ ), 3.978 (s, 3H; CH), 3.940 (s, 3H; CH), 3.853 (s, 4H; CH), 3.846 (s, 4H; CH), 3.843 (s, 4H; CH), 3.830 (s, 4H; CH), 3.822 (s, 4H; CH), 3.802 (s, 4H; CH), 3.056–3.089 (m, 3H; CH), 2.410 (t, 3H; CH,  $J = 6.80$ ), 2.385 (t, 3H; CH,  $J = 2.04$ ), 2.238 (t, 3H; CH,  $J = 7.12$ ), 2.289 (t, 6H; CH,  $J = 7.12$ ), 2.250 (t, 3H; CH,  $J = 7.12$ ), 2.211 (t, 3H; CH,  $J = 7.48$ ), 1.812 (t, 2H; CH,  $J = 7.48$ ), 1.580 (t, 2H; CH,  $J = 7.12$ ), 0.854 (t, 1H; CH,  $J = 7.12$ ). ESI-TOF-Mass:  $m/z$  calcd. C<sub>84</sub>H<sub>96</sub>N<sub>24</sub>O<sub>13</sub> [M+2H]<sup>+</sup> 1650.7589; found 1650.8668.

### Photoirradiation conditions and HPLC analysis

Samples were photoirradiated on a transilluminator at 365 nm for 15 min on ice unless otherwise stated.

Reaction mixtures contained 10 mM cacodylate buffer (pH 7.0), 200 mM isopropanol, 4  $\mu\text{M}$  DNA, with 4  $\mu\text{M}$  PPI, polyamide or pyrene butyric acid. Photoirradiated samples were injected to HPLC using the PU-980 HPLC system (Jasco) with a Chemcobond 5-ODS-H column. Detection was carried out at 254 nm. Elution was with 0.05 M ammonium formate containing 3–10% acetonitrile in a linear gradient for 40 min. In Figure 3, the yield and conversion were determined by comparing the substrates or products before and after photoirradiation. The complementary strand, the peak of which did not change by photoirradiation, was used as a standard peak.

### Polyacrylamide gel electrophoresis analysis

Oligonucleotides were labelled with FITC at the 5' end. The photoirradiated DNAs containing uracil were cleaved via treatment with two units of Uracil DNA Glycosylase, UNG (Takara) for 1 h at 37°C, followed by heat treatment at 95°C for 10 min with 0.1 M NaOH. After addition of 4  $\mu\text{l}$  of loading buffer (95% formamide, 0.1% xylene cyanol and 20 mM ethylenediaminetetraacetic acid), the samples were loaded onto 20% polyacrylamide gels in Tris borate buffer (pH 8.3) containing 7 M urea. After electrophoresis at 200 V for 100 min, the gels were analysed using FLA-3000 (Fujifilm).

## RESULTS

### Synthesis of PPI

PPI was synthesized via coupling between a pyrene succinimide ester and a PI polyamide via solid-phase synthesis (Figure 1). After completion of machine-assisted

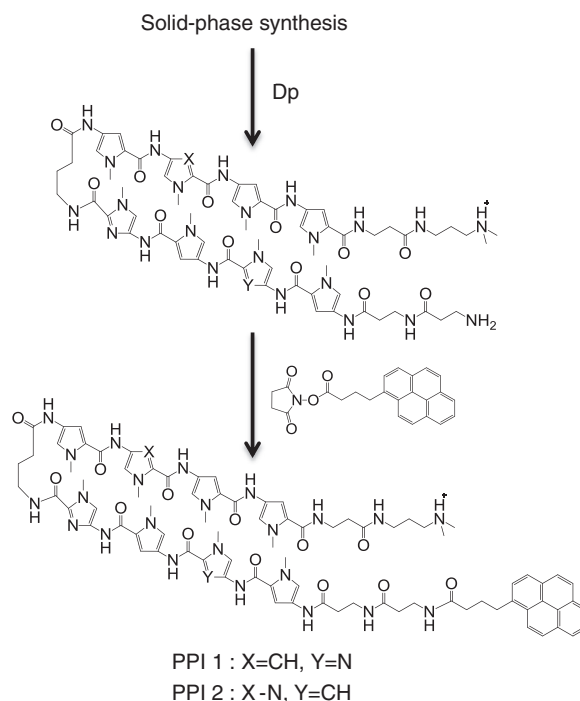


Figure 1. Syntheses of PPI 1 and PPI 2.

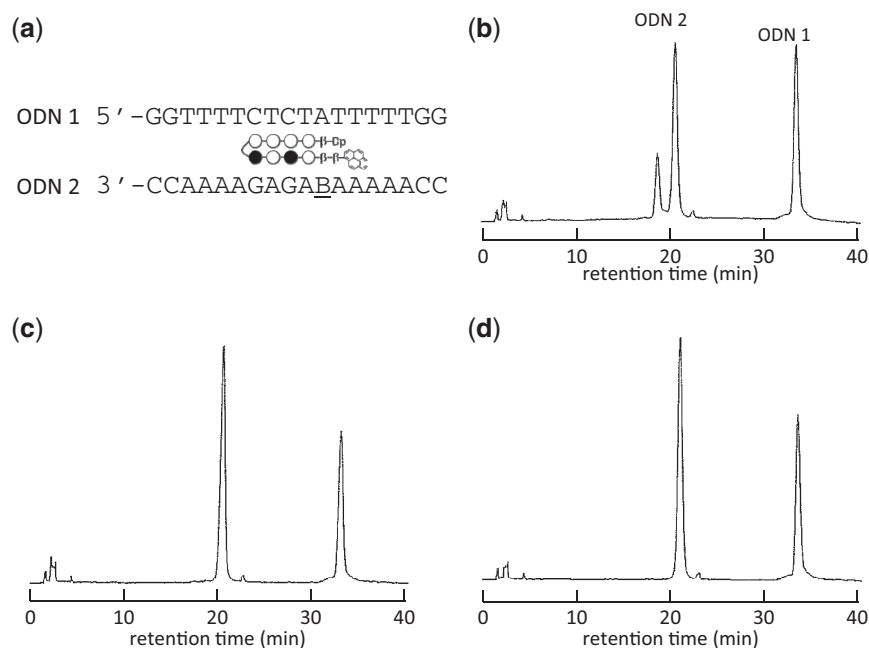
solid-phase synthesis, the hairpin PI polyamides containing amine at the N terminal were cleaved from resins using *N,N*-dimethyl-1,3-propanediamine in an Eppendorf Thermomixer at 55°C for 3 h. After the solvent was removed *in vacuo*, the resulting crude extract was dissolved in *N,N*-Dimethylformamide (DMF), diisopropylethylamine and an excess amount of the pyrene succinimide ester. The coupling reaction was completed within 1 h in quantitative yield. The products were purified by HPLC and confirmed using ESI-TOF-MS and <sup>1</sup>H-NMR analysis.

### Photoinduced electron injection from PPI into DNA

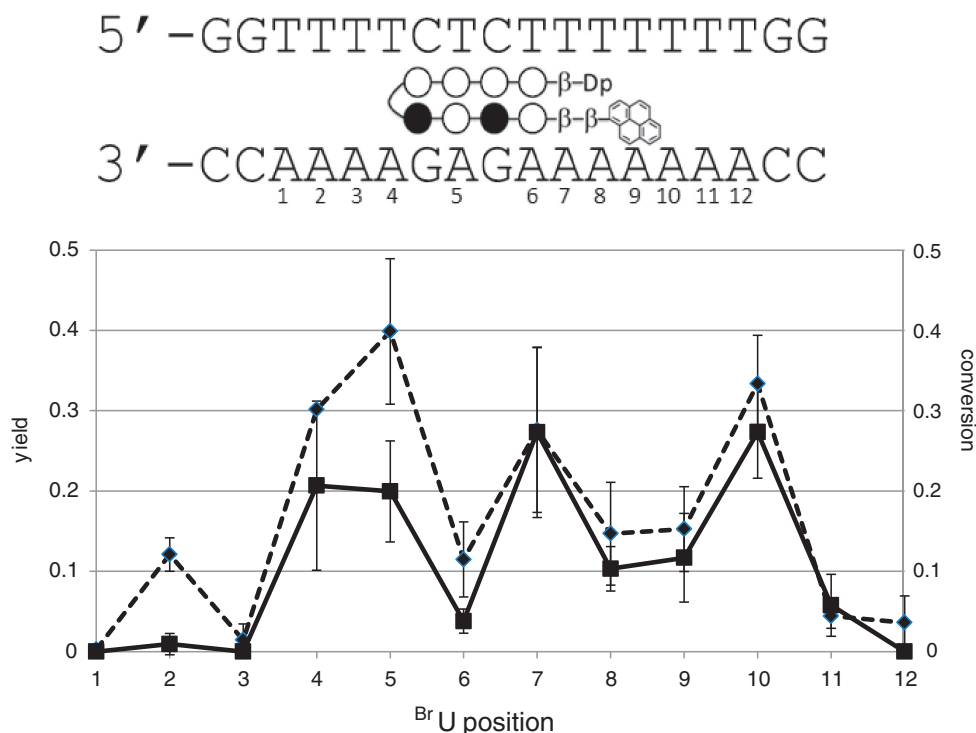
We examined the intermolecular electron-injection ability of PPI 1 using 5-BrU-containing ODN 1 with complementary ODN 2 in the presence of 0.2 M isopropanol (Figure 2a). HPLC analysis of the reaction mixture showed that one peak appeared after photoirradiation (Figure 2b). This peak was identified as a uracil-containing product, which was confirmed by MS analysis and cleavage using UNG and subsequent heat treatment in alkali (see 'Materials and Methods' section and Supplementary Figure S1). The uracil-containing product was not obtained by photoirradiation of pyrene or PI polyamide moieties in the presence of ODNs (Figure 2c and d). These results indicate that the uracil-containing product was formed by electron injection from PPI. Photoirradiation of ODNs 1 and 2 with PPI 1 in the absence of 0.2 M isopropanol provided many products because of hydrogen abstraction from the deoxyribose backbone by the uracil-5-yl radical.

### Range of photoinduced electron injection from PPI into DNA

To investigate the range of efficient electron injection, 12 kinds of ODNs containing 5-BrU at different positions were designed, and their photoreactivity in the presence of PPI 1 was investigated. In each ODN, one A–T base pair was replaced with a 5-BrU–A base pair at the indicated position (Figure 3, upper panel). PPI 1 was shown to bind to each ODN, which was confirmed by the increase of T<sub>m</sub> value (Supplementary Figure S2 and Supplementary Table S1). Electron-transfer activities were measured by HPLC as the consumption of 5-BrU-containing ODN or as the amount of the reduced product after photoirradiation. As shown in Figure 3, efficient electron transfer was observed when 5-BrU was introduced at positions 4–11. These results indicate that electron injection can be achieved within a range of ~8 bp from the binding site of PPI. The different reactivities observed may be explained by differences in the efficiency of electron transfer, structure or sequence context. Among them, the distance from electron donor and acceptor is important for reactivities. The reactivity of BrU may be decided by the balance between the ratio of electron transfer and back electron transfer. This may explain why there is no simple correlation between the distance and reaction yield. In almost all cases, the products were only reduced products; however, in the case of ODNs containing 5-BrU at position 5, several different products were observed (data not shown).



**Figure 2.** HPLC analysis of electron transfer into DNA from intermolecular PPIs. ODN 1 and ODN 2, which contain 5-BrU (a), were photoirradiated for 15 min with PPI 1 (b), the precursor of PPI 1, which lacks pyrene (c) or pyrene butyric acid (d). The photoirradiated samples were injected into HPLC with 50 mM ammonium formate containing 3–10% acetonitrile in a linear gradient for 40 min at 40°C. B, 5-BrU; ○, pyrrole; ●, imidazole; β, β-alanine; (∶) γ-aminobutyric acid.



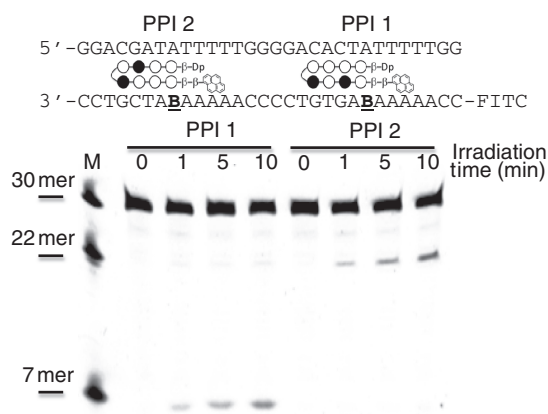
**Figure 3.** Determination of the range of efficient electron transfer from PPI. Twelve kinds of dsDNAs were designed by replacing one adenine-thymine pair with a 5-BrU-adenine pair. The sequence and position of 5-BrU are shown in the upper panel of the figure. Reaction rates were calculated by the consumption of the starting 5-BrU-containing oligonucleotide (dashed line) or by the amount of reduced products (solid line), which were measured using HPLC.

### Sequence-specific photoinduced electron injection by PPI into DNA

To examine the sequence selectivity of the photoinduced electron injection by PPI, the electron-injection activities of PPI 1 and PPI 2 were investigated using an FITC-labelled ODN containing two 5-BrU residues. The structure and target site of PPI 1 and PPI 2 are shown in Figure 4. After photoirradiation, the resulting uracil-containing products were cleaved at the uracil site using UNG and were analysed via gel electrophoresis. It was shown that both polyamides selectively injected electrons into individual target 5-BrU residues (Figure 4). These results indicate that designed PPIs can selectively inject electrons into target sites in DNA. Previously, we demonstrated that sso7d, which is a non-sequence-specific DNA-binding protein of Archaea, injected electrons into 5-BrU and a thymine photodimer in DNA under photoirradiation (24,25). Taken together, these results suggest that sequence-specific electron transfer into DNA from an intermolecular electron donor is possible, supporting the possibility that PPIs can be used for the elucidation of the possible biological roles of electron transfer into DNA.

### CONCLUSION

In the present study, we demonstrated that the designed PPIs were able to sequence selectively inject electrons into



**Figure 4.** Site-specific electron injection into DNA. An oligonucleotide containing two 5-BrU residues and FITC was prepared and photoirradiated with PPI 1 or PPI 2 for the indicated time on ice. The resulting samples were cleaved at the uracil site using UNG following heat treatment and loaded onto gels. A 7-mer, 22-mer and 30-mer ODNs were used as a size marker.

DNA under photoirradiation conditions, which was confirmed by the reduction of 5-BrU into uracil. Efficient electron injection from PPI was observed in ~7 bp from the binding site of PPI. As PPIs can be designed and synthesized with a predetermined and defined sequence, PPIs can be used for the elucidation of the possible biological roles of electron transfer into DNA. In the field of

engineering, PPI can be applied to the development of DNA-based electronic nanodevices. Recently, there has been growing interest in developing DNA-based nanodevices using its self-assembling and molecular-reorganization properties. One of the most important aspects of a DNA-based nanodevice is its ability to change its shape or function in response to an external stimulus, such as a small molecule, a protein or other DNA molecules. Site-specific electron transfer by PPI may become a suitable stimulus for DNA-based electronic nanodevices.

## SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online: Supplementary Table 1 and Supplementary Figures 1 and 2.

## FUNDING

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