

Thiazole orange-conjugated peptide nucleic acid for fluorescent detection of specific DNA sequence and site-selective photodamage

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ABSTRACT:

A fluorescent probe to detect specific sequence in double-stranded DNA (dsDNA) has been synthesized by conjugating thiazole orange (TO) with pseudo-complementary peptide nucleic acid (pcPNA). A pair of TO-conjugated pcPNAs bound to the target site in dsDNA through double-duplex invasion, and promoted intercalation of the TO moieties for notable enhancement of their fluorescence. Site-specificity of the detection is high enough to differentiate only one base-pair difference in DNA. These TO-pcPNA conjugates also function as site-selective photo-damage inducer. Upon irradiation of visible light, singlet oxygen was generated by energy transfer from the dyes at the invasion site, resulting in site-selective formation of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxoG) there.

Introduction

Thiazole orange (TO) emits strong fluorescence, when it binds to single-stranded DNA (ssDNA) or intercalates to double-stranded DNA (dsDNA). Otherwise, the dye is almost silent due to energy dissipating rotation between the benzothiazole and the quinoline.^{1,2} Therefore, various TO-based probes have been already synthesized to detect DNA or RNA.³⁻¹⁴ In most cases, dsDNA was first denatured at elevated temperatures and the resultant ssDNA was subjected to the probes. Accordingly, the pioneering work by Dervan's group was attractive in that no denaturation process was required for the detection. When pyrrole-imidazole polyamide bearing a TO bound to minor groove at specific sequence of dsDNA, the TO intercalated to the DNA and efficiently emitted fluorescence.¹⁴ Recently, triplex-forming oligonucleotides were labeled with two TO units and used for exciton-controlled hybridization-sensitive fluorescent detection.¹⁰ In addition to these fluorescent properties, TO acts as a photo-induced generator of singlet oxygen ($^1\text{O}_2$) by transferring the energy from its triplet state to ground state oxygen.¹⁵⁻¹⁷ The $^1\text{O}_2$ formed in turn induces damages in dsDNA through oxidation of nucleobases. Especially, guanine is most easily oxidized to form 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxoG).^{18,19}

Pseudo-complementary peptide nucleic acid (pcPNA) is composed of poly[*N*-aminoethylglycine] backbone, in place of poly[deoxyribose-phosphodiester] linkage in DNA. Furthermore, conventional nucleobases A and T are replaced by 2,6-diaminopurine (D) and 2-thiouracil (U), respectively. Importantly, a pair of pcPNAs recognizes a predetermined sequence in dsDNA through so-called double-duplex invasion.²⁰⁻²² There, each pcPNA binds to the corresponding complementary DNA strand according to the Watson-Crick rule. It should be noteworthy that there is no critical limitation in the choice of target sequence. By combining this phenomenon with Ce(IV)/EDTA complex as molecular scissors, dsDNA was selectively cut at target site.²³⁻²⁵

In this paper, we prepare the conjugates of a TO dye with pcPNA, and combine two of them for dual functions as (i) fluorescent detector of specific DNA and (ii) site-selective photodamage inducer. Upon double-duplex invasion of the pcPNAs in the conjugates to predetermined positions in dsDNA, the TO groups intercalate to the DNA near there. Thus, DNA fragments having a specific sequence are precisely and selectively detected by the fluorescence from the two TO dyes. Furthermore, under the irradiation of visible light, the $^1\text{O}_2$ formed by the photosensitization of the TO groups preferentially attacks the neighboring guanines, resulting in site-selective formation of 8-oxoG on both strands of DNA at the invasion site.

Results and discussion

The sequences of TO-conjugated pcPNAs, TO-pcPNA1/TO-pcPNA2, and of their DNA target site are presented in Fig. 1a. All the substrate DNAs (100 bp, 381 bp, or a plasmid pBFP-N1) contain either the whole or a part of the gene for blue fluorescent protein (BFP). The pcPNA1 and pcPNA2 strands were designed to invade these DNA substrates at the coding site of the BFP chromophore. A TO dye was conjugated to the *N*-termini of these pcPNAs via a PEG spacer (17 atoms and 19.2 Å); Fig. 1b. Because the affinity of TO to dsDNA is stronger than that to ssDNA,² the invasion of TO-pcPNA1/TO-pcPNA2 should lead to the intercalation of the two TO moieties to the adjacent sites in the dsDNA, as schematically depicted in Fig. 1c.

Fluorescent detection of target sequence in dsDNA by TO-conjugated pcPNA

In Fig. 2, the activity of TO-pcPNA1/TO-pcPNA2 combination to detect a specific DNA sequence was investigated. The effect of DNA^{BFP} (381 bp) involving a part of the BFP gene was compared with that of DNA^{EGFP} for enhanced green fluorescent protein (EGFP). These two genes are homologous and different only in their chromophore-coding regions; -AGTCATGGT- (Ser65-His66-Gly67) for BFP and -ACCTACGGC- (Thr65-Tyr66-Gly67) for EGFP.²⁶ The other parts in these two DNAs are almost the same as each other. In the presence of DNA^{BFP}, the fluorescence from the two conjugates was very strong (blue line in Fig. 2a). The TO-pcPNA1/TO-pcPNA2 combination binds to the chromophore-coding region of BFP, and promotes the intercalation of the TOs to the DNA to enhance the emission of their fluorescence. With the use of DNA^{EGFP} in place of DNA^{BFP}, however, the fluorescence was far weaker (green line). Apparently, the subtle difference between the sequences of these two DNAs (at the chromophore sites) is clearly differentiated by the present pcPNA double-duplex invasion system.

The plasmid involving the whole BFP gene (pBFP-N1) also enhanced the fluorescence in a greater magnitude than pEGFP-N1 involving the EGFP gene (Fig. 2b). These two homologous plasmids were also explicitly differentiated by TO-pcPNA1/TO-pcPNA2 combination. In this case, however, even non-complementary pEGFP-N1 promoted the fluorescence to considerable extent, decreasing the ratio of differentiation between pBFP-N1 and pEGFP-N1. Non-selective binding of pEGFP-N1 with TO-pcPNA1 and/or TO-pcPNA2 is not negligible here, due to the larger off-target size of plasmid. The binding constant for the intercalation of a TO dye into linear dsDNA is $\sim 10^5 \text{ M}^{-1}$,² and thus direct interaction of the TO moieties of the conjugates with the dsDNA should

be minimal under the conditions employed ($[dsDNA] = 25 \text{ nM}$ and $[each \text{ TO-conjugated pcPNA}] = 25 \text{ nM}$).

Selective detection of target fragments in electrophoresis gels.

The TO-pcPNA1/TO-pcPNA2 combination also successfully detects a specific sequence even in agarose gels used for electrophoresis. In Fig. 3a, pBFP-N1 plasmid was cut by various restriction enzymes (Stu I, Pvu II, Dra I/Apa I, or Hae II), after it formed the invasion complex with the TO-pcPNA1/TO-pcPNA2 combination. The products were directly (without any staining) analyzed by agarose gel electrophoresis with the use of SYBR Green method (473 nm/520 nm) of a GE healthcare Typhoon FLA 7000 imaging analyzer. SYBR Green has about the same excitation and emission wavelength range of TO. Although several fragments of different lengths should be formed in the solution for each lane (Fig. 3c and d), only the fragments containing the target sequence were detected under the present non-staining conditions (4.7 kbp in lane 1, 4.1 kbp in lane 2, 0.8 kbp in lane 3, and 0.3 kbp in lane 4 in Fig. 3a).

After the experiments in Fig. 3a, the gel was stained with SYBR Safe (Fig. 3b). As expected, all the fragments listed in Fig. 3c were detectable. Apparently, the TO-pcPNA1/TO-pcPNA2 remains bound to the target sequence in the dsDNA even in the agarose gels, allowing the fluorescent detection of only the target fragments. Thus, the invasion complexes can be formed just prior to the electrophoresis. This method should be useful to pin down target fragments in electrophoresis gels, when their sizes are not known.

TO-conjugated pcPNA for fluorescent detection of a mismatch in dsDNA.

The mismatch-discrimination ability of the TO-pcPNA1/TO-pcPNA2 combination was investigated using four kinds of 100 bp DNAs (Fig. 4a). In ds1, ds2, ds3, and ds4, one base-pair at the target site was systematically changed. In the presence of fully matching ds1, the fluorescence from the TO-pcPNA1/TO-pcPNA2 combination is very strong due to efficient intercalation of the TOs to the DNA (black line in Fig. 4b). With the use of mismatching ds2, ds3, or ds4, however, the emission height is less than one fifth (blue, red and green lines). Highly strict mismatch recognition by the present system is evident. The double-duplex invasion is efficient only when both of two pcPNAs are completely complementary with the DNA strands, and otherwise severely deteriorated.²⁷ Note that, when one base-pair in the DNA is changed to another, one mismatch is introduced between TO-pcPNA1 and the counterpart DNA strand, and

another mismatch is introduced between TO-pcPNA2 and the corresponding counterpart.

Site-selective photodamage of dsDNA using TO-conjugated pcPNA

In the invasion complex of plasmid pBFP-N1 with TO-pcPNA1/TO-pcPNA2, the two TO groups are located, through intercalation, near several guanine residues on both DNA strands (Fig. 5). These guanines are primarily oxidized by the $^1\text{O}_2$, generated under photoirradiation through the sensitization of the TO groups, and are mainly converted to 8-oxoG.^{18,19} First, the invasion complex of pBFP-N1 was irradiated at 500 nm for 30 min under air. Then, the formation of 8-oxoG in the DNA was assessed by treating the photoproduct with bacterial formamidopyrimidine DNA *N*-glycosylase (Fpg). Prior to this Fpg treatment, the TO-conjugated pcPNAs were completely removed from the DNA products (and the DNA substrates) as described in Experimental section. This enzyme recognizes 8-oxoG in dsDNA and cuts the DNA strand through removal of the oxidized nucleobase.²⁸ Accordingly, if 8-oxoG groups are formed in both strands of pBFP-N1 in a sufficiently close proximity, the Fpg treatment of the photo-product should lead to double-strand break in this dsDNA. In case that the 8-oxoG on one strand is far from that on another strand, however, nick structures should be formed at the corresponding positions rather than the double-strand break (*vide infra*).

In order to determine the site of this double-strand break (and thus the positions of 8-oxoGs) in pBFP-N1, the product of the Fpg treatment was further digested by a restriction enzyme *Stu* I, and analyzed by 1.0 % agarose gel electrophoresis. As presented in lane 2 in Fig. 5, two scission bands were formed at around 1.7 and 3.0 kbp. These are exactly the fragments formed when pBFP-N1 is cut at both the invasion site of TO-pcPNA1/TO-pcPNA2 and the *Stu* I site.²⁹ Thus, it has been confirmed that, upon the irradiation at 500 nm, 8-oxoGs are formed at the double-duplex invasion site on both strands of pBFP-N1. Note that the positions of these 8-oxoGs in both strands must be sufficiently close to each other in order to form the double-strand break. As expected, no scission bands were detected without the photoirradiation (lane 1). Eminence of TO-conjugated pcPNA as site-selective photodamage inducer has been evidenced.

The efficiency of the formation of the 1.7 and 3.0 kbp fragments was slightly increased, when the Fpg products were further treated with T7 endonuclease I before the *Stu* I scission (lane 4). This enzyme cuts nick structures in dsDNA, so that this increase would correspond to the photo-products in which 8-oxoG is formed on only one DNA strand and another strand remains intact (or 8-oxoG formed on one strand is far from

that on another strand). The Fpg treatment of these products should provide nick structures at the 8-oxoG sites, which are then converted to double-strand break by the following treatment with T7 endonuclease I.

Conclusions

By conjugating TO with pcPNA and combining two of these conjugates, a useful fluorescent probe for a specific sequence in dsDNA has been devised. This probe satisfactorily recognizes small difference in DNA sequences, and no denaturation procedure of dsDNA is necessary. Even a tiny target site in the plasmid DNA could be well detected by the fluorescence from this probe. The fluorescence from the TO groups is also clearly observable in electrophoresis gels, allowing direct detection of desired fragments there. Moreover, these conjugates induce site-selective photodamage in dsDNA through the TO-sensitized generation of $^1\text{O}_2$. 8-OxoG is the primary photodamage product. This method would make it possible to investigate the effect of oxidative damage at selective site *in vivo*. In both of these two applications, the target DNA sequence can be rather freely chosen, and the conjugates are intrinsically stable against various enzymes. Thus, the present probe would be a useful tool for new biotechnology.

Experimental section

Materials

The pcPNAs were synthesized using standard Boc-chemistry-based solid phase peptide synthesis using the PNA monomers obtained from ASM research Chemicals and Panagene.²³ In order to facilitate their invasion to dsDNA, three lysine residues were attached to them. t-Boc-N-amido-dPEG®₄-acid (for the PEG spacer) was purchased from Quanta BioDesign. The TO-conjugated pcPNAs were prepared using 2-[[1-(5-carboxypentyl)-4(1H)-quinolinyldiene]methyl]-3-methylbenzothiazolium bromide, which was synthesized according to the literature.³⁰ The conjugates were purified by reversed-phase HPLC, and characterized by UV-visible spectroscopy and MALDI-TOF mass spectrometry (see ESI).

A plasmid substrate pBFP-N1 (4733 bp) containing a BFP gene was prepared in a conventional manner.³¹ The linear DNA coding a part of the gene for BFP or EGFP (381 bp) was obtained by PCR. The 100 mer oligonucleotides ds1-4 were prepared by annealing equimolar amounts of the corresponding DNA complements (from Operon). The enzymes to treat the photoproducts of the invasion complex (Fpg and T7 endonuclease I) were purchased from New England Biolabs.

Fluorescence spectroscopy.

The spectra were recorded in pH 7.0 HEPES buffer (5.0 mM) on a JASCO FP-6500 spectrofluorometer at room temperature (22°C). Excitation wavelength was 500 nm, and 1 cm path length cell was used.

Gel electrophoresis.

The electrophoresis was achieved on 1.0 % agarose gel. The products of the enzymatic scission in the presence of TO-pcPNA1/TO-pcPNA2 were detected without any staining in terms of the emission from the TO groups by using SYBR Green method (473 nm/520 nm) of an imaging analyzer Typhoon FLA 7000 (GE healthcare). Otherwise, the bands were stained by GelStar (Lonza) or SYBR Safe (Life Technologies).

Assay of site-selective DNA damage.

The pBFP-N1 plasmid was first mixed with TO-pcPNA1/TO-pcPNA2 in pH 7.0 HEPES buffer containing 20 mM NaCl, and the mixture was incubated overnight at 50°C for invasion complex formation. Aliquots were then irradiated at 500 nm under air ([pBFP-N1] = 4.6 nM, [each TO-conjugated pcPNA] = 25 nM, [HEPES (pH 7.0)] = 5.0 mM and [NaCl] = 20 mM). The monochromatic light was obtained with a Xe lamp (300 W; Asahi Spectra Co. Ltd.; MAX-303) equipped with high concentrating lens unit (ϕ 2.5), a band-pass filter MX0500 for 500 nm and a ND filter.

After the photoirradiation, NaCl was added to final concentration of 0.5 M, and the sample solutions were incubated at 50°C for 3 h to remove TO-conjugated pcPNAs from the invasion complex (these purification conditions were determined by independent experiments using 381 bp DNA; see Fig. S3 for details). Then the sample solutions were purified using QIAquick PCR Purification Kit (QIAGEN) and incubated with Fpg enzyme at 37°C for 16 h. The reaction mixture was treated with Stu I, and then was subjected to 1.0 % gel electrophoresis. In case that the Fpg product was further treated with T7 endonuclease I, this enzyme was added after Fpg was denatured at 60°C for 15 min and the mixture was purified using QIAquick PCR Purification Kit. The product was then digested by Stu I, and analyzed by the gel electrophoresis.

Electronic supplementary information (ESI) available:

MALDI-TOF MS spectra, absorption spectra of TO-conjugated pcPNAs, gel-shift assay for the invasion complex formation, and the sequence of pBFP-N1. See DOI:

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REFERENCES

- 1 C. C. Trevithick-Sutton, L. Mikelsons, V. Filippenko and J. C. Scaiano, *Photochem. Photobiol.*, 2007, **83**, 556-562.
- 2 J. Nygren, N. Svanvik and M. Kubista, *Biopolymers*, 1998, **46**, 39-51.
- 3 N. Svanvik, G. Westman, D. Wang and M. Kubista, *Anal. Biochem.*, 2000, **281**, 26-35.
- 4 E. Socher, A. Knoll and O. Seitz, *Org. Biomol. Chem.*, 2012, **10**, 7363-7371.
- 5 O. Seitz, F. Bergmann and D. Heindl, *Angew. Chem. Int. Ed. Engl.*, 1999, **38**, 2203-2206.
- 6 D. V. Jarikote, N. Krebs, S. Tannert, B. Roder and O. Seitz, *Chem.-Eur. J.*, 2007, **13**, 300-310.
- 7 L. Bethge, D. V. Jarikote and O. Seitz, *Bioorg. Med. Chem.*, 2008, **16**, 114-125.
- 8 Y. Kam, A. Rubinstein, A. Nissan, D. Halle and E. Yavin, *Mol. Pharm.*, 2012, **9**, 685-693.
- 9 A. Okamoto, *Chem. Soc. Rev.*, 2011, **40**, 5815-5828.
- 10 S. Ikeda, H. Yanagisawa, M. Yuki and A. Okamoto, *Artificial DNA: PNA & XNA*, 2013, **4**, 19-27.
- 11 S. Ikeda, T. Kubota, K. Kino and A. Okamoto, *Bioconjugate Chem.*, 2008, **19**, 1719-1725.
- 12 S. Berndl, M. Breunig, A. Gopferich and H. A. Wagenknecht, *Org. Biomol. Chem.*, 2010, **8**, 997-999.
- 13 F. Menacher, M. Rubner, S. Berndl and H. A. Wagenknecht, *J. Org. Chem.*, 2008, **73**, 4263-4266.

- 14 E. J. Fechter, B. Olenyuk and P. B. Dervan, *J. Am. Chem. Soc.*, 2005, **127**, 16685-16691.
- 15 K. P. Mahon, Jr., R. F. Ortiz-Meoz, E. G. Prestwich and S. O. Kelley, *Chem. Commun.*, 2003, 1956-1957.
- 16 K. P. Mahon, Jr., M. D. Roy, J. R. Carreon, E. G. Prestwich, J. L. Rouge, S. Shin and S. O. Kelley, *ChemBioChem*, 2006, **7**, 766-773.
- 17 A. Biton, A. Ezra, J. Kasparkova, V. Brabec and E. Yavin, *Bioconjugate Chem.*, 2010, **21**, 616-621.
- 18 C. Burrows and J. G. Muller, *Chem. Rev.*, 1998, **98**, 1109-1151.
- 19 J. Cadet, S. Mouret, J. L. Ravanat and T. Douki, *Photochem. Photobiol.*, 2012, **88**, 1048-1065.
- 20 J. Lohse, O. Dahl and P. E. Nielsen, *Proc. Natl. Acad. Sci. U.S.A.*, 1999, **96**, 11804-11808.
- 21 K. I. Izvolsky, V. V. Demidov, P. E. Nielsen and M. D. Frank-Kamenetskii, *Biochemistry*, 2000, **39**, 10908-10913.
- 22 E. Protozanova, V. V. Demidov, P. E. Nielsen and M. D. Frank-Kamenetskii, *Nucleic Acids Res.*, 2003, **31**, 3929-3935.
- 23 M. Komiyama, Y. Aiba, T. Ishizuka and J. Sumaoka, *Nat. Protoc.* 2008, **3**, 646-654.
- 24 Y. Aiba, J. Sumaoka and M. Komiyama, *Chem. Soc. Rev.* 2011, **40**, 5657-5668.
- 25 K. Ito, H. Katada, N. Shigi and M. Komiyama, *Chem. Commun.* 2009, 6542-6544.
- 26 For the sequences of BFP and EGFP near the chromophore-coding regions; H. Katada, T. Harumoto, N. Shigi and M. Komiyama, *Nucl. Acids Res.*, 2012, **40**, e81.
- 27 Similar results have been previously reported; Y. Miyajima, T. Ishizuka, Y. Yamamoto, J. Sumaoka and M. Komiyama, *J. Am. Chem. Soc.*, 2009, **131**, 2657-2662.
- 28 Y. Osakada, K. Kawai, T. Tachikawa, M. Fujitsuka, K. Tainaka, S. Tero-Kubota and T. Majima, *Chem.-Eur. J.*, 2012, **18**, 1060-1063.
- 29 H. Katada, J. C. Hsuan, N. Shigi and M. Komiyama, *Chem. Commun.* 2009, 6545-6547.
- 30 J. R. Carreon, K. M. Stewart, K. P. Mahon, Jr., S. Shin and S. O. Kelley, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 5182-5185.
- 31 H. Katada and M. Komiyama, *Chembiochem*, 2009, **10**, 1279-1288.

5' TGG CCC ACC CTC GTG ACT ACT CTT AGT CAT GGT GTA CAG T 3'
NH₂-KK UGD UGD GDD UCD GUD K-TO
TO-pcPNA1

TO-pcPNA2
TO-KUC GUG DCU DCU CUU DKK-NH₂
3' ACC GGG TGG GAG CAC TGA TGA GAA TCA GTT CCA CAT GTC A 5'

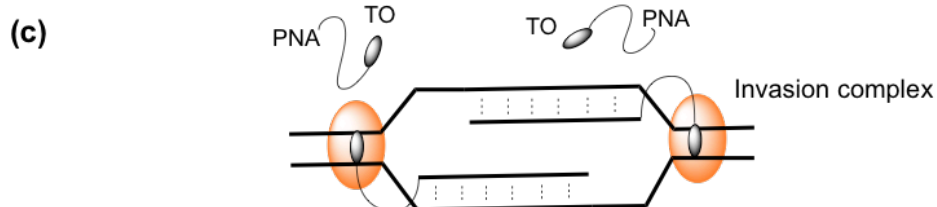
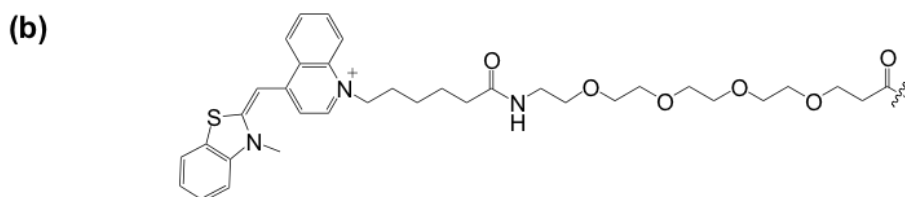
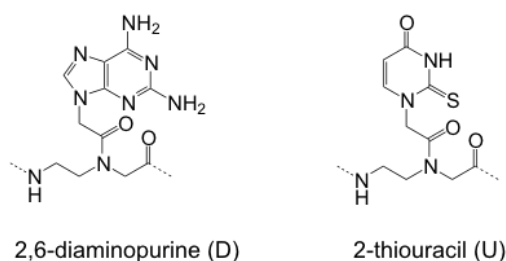


Fig. 1 (a) The sequences of target site in substrate DNA and TO-conjugated pcPNAs (TO-pcPNA1/TO-pcPNA2) with the structures of pseudo-complementary bases D and U. (b) Chemical structures of TO (thiazole orange) and PEG linker. (c) Schematic representation of invasion complex between dsDNA and TO-conjugated pcPNAs. The two TO moieties should intercalate to the adjacent sites in the dsDNA.

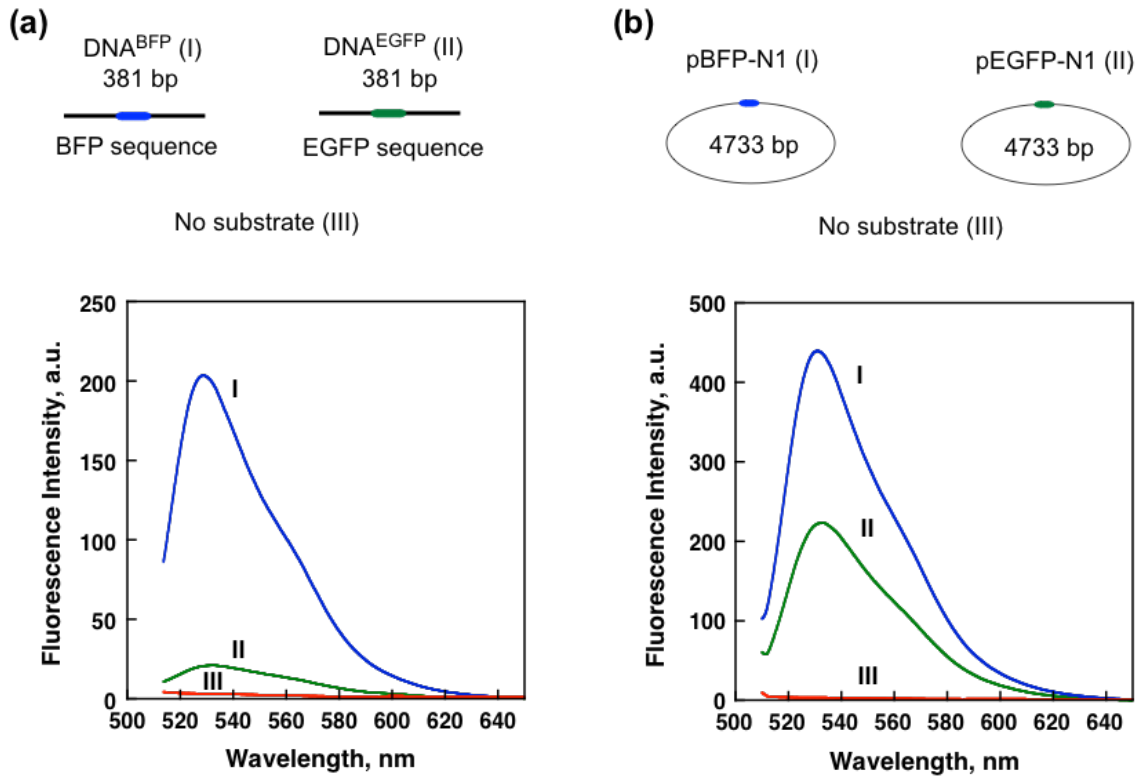


Fig. 2 (a) Fluorescence spectra of the TO-pcPNA1/TO-pcPNA2 combination in the presence of 381bp dsDNA containing BFP sequence (blue, I), 381bp dsDNA containing EGFP sequence (green, II), or no DNA (red, III). Excitation wavelength is 500 nm. In (b), pBFP-N1 plasmid (blue, I) and pEGFP-N1 plasmid (green, II) are used, in place of the 381 bp dsDNAs. The spectrum with no DNA(III) is shown in red. Invasion complexes were prepared by overnight incubation at 50°C under the conditions: [dsDNA] = 25 nM and [each TO-conjugated pcPNA] = 25 nM in pH 7.0 HEPES buffer (5.0 mM) containing 50 mM NaCl.

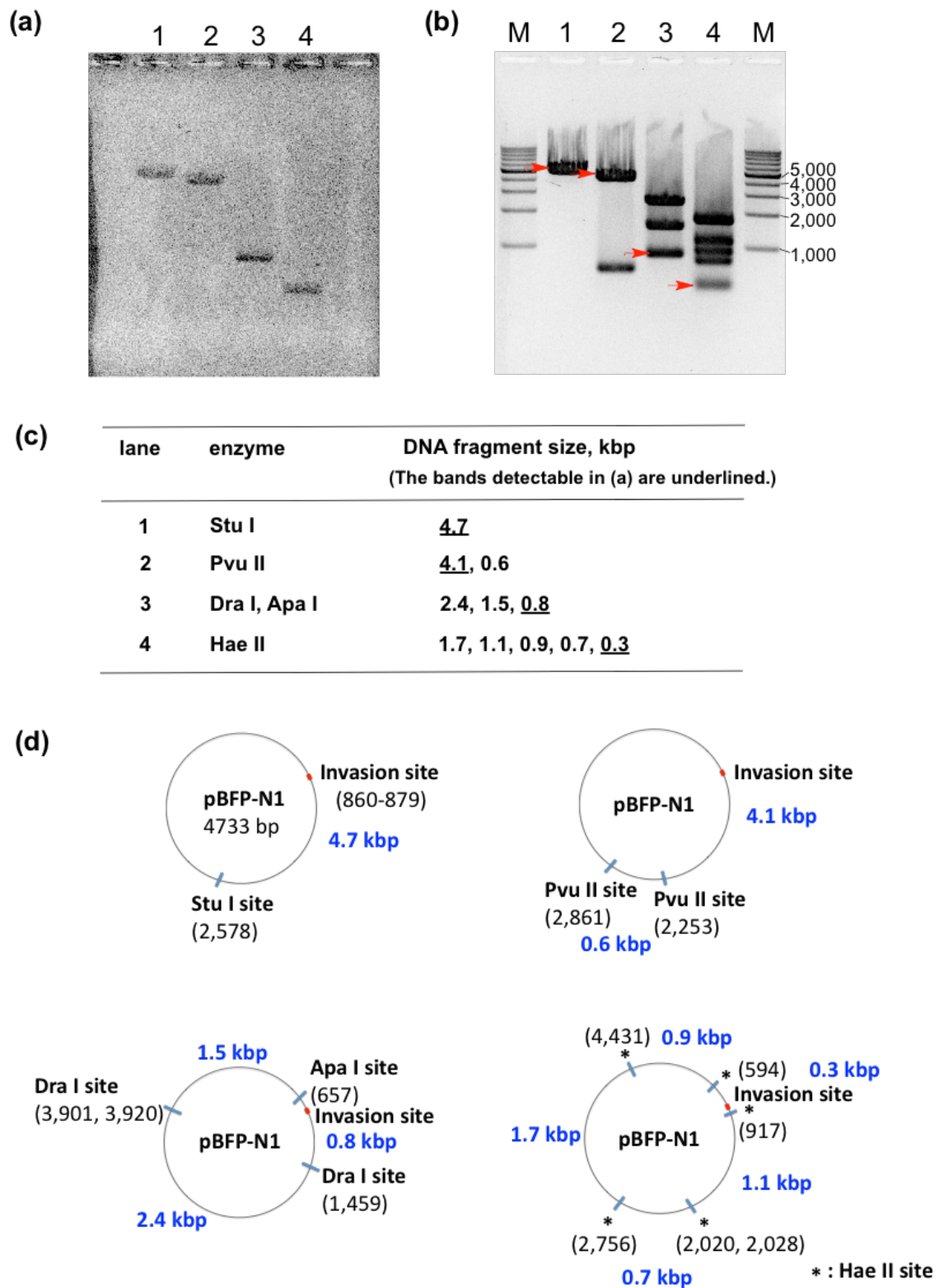


Fig. 3 (a) Selective detection of target fragments in electrophoresis gel using TO-conjugated pcPNA. The invasion complex of pBFP-N1 with

TO-pcPNA1/TO-pcPNA2 was treated with Stu I (lane 1), Pvu II (lane 2), Dra I/Apa I (lane 3), or Hae II (lane 4), and analyzed by agarose gel electrophoresis. The bands were detected by SYBR Green method (473 nm/520 nm) without staining. Note that only the fragments containing the invasion site (underlined in (c)) are detected. When the gel was then stained with SYBR Safe, however, all the expected fragments were detected in lanes 1-4 in (b). The scission site of each enzyme in pBFP-N1 is shown in (d) (more details should be referred to ESI).

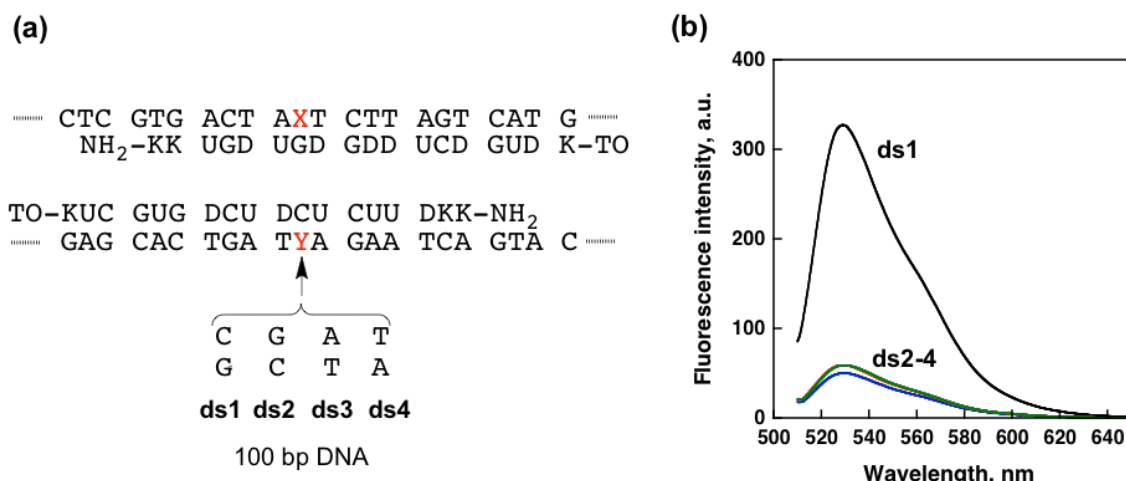


Fig. 4 (a) The sequences of DNA substrates ds1-4 (100 bp). (b) Fluorescence spectra of the TO-pcPNA1/TO-pcPNA2 combination in the presence of fully matching DNA (ds1, black line) or mismatching DNAs (ds2, blue; ds3, red; ds4, green). The profile of ds3 overlaps with that of ds4. Excitation wavelength is 500 nm. Invasion complexes were prepared by overnight incubation at 50°C under the conditions: [DNA duplex] = 25 nM and [each TO-conjugated pcPNA] = 25 nM in pH 7.0 HEPES buffer (5.0 mM) containing 50 mM NaCl.

