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Sequence-specific Hydrolysis of Single-stranded DNA by PNA-Cerium (IV) Adduct

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Abstract: A novel artificial site specific cleavage reagent, with peptide nucleic acid (PNA) as sequence-recognizing moiety and cerium (IV) ions as "scissors" for cleaving target DNA, was synthesized. Subsequently, it was employed in the cleavage of target 26-mer single-stranded DNA (ssDNA), which has 10-mer sequence complementary with PNA recognizer in the hybrids, under physiological conditions. Reversed-phase high-performance liquid chromatogram (RP-HPLC) experiments indicated that the artificial site specific cleavage reagent could cleave the target DNA specifically.

Keywords: Peptide nucleic acid (PNA), artificial site specific cleavage reagent, hydrolysis, reversed-phase high-performance liquid chromatogram (RP-HPLC).

Sequence-specific recognition and site-specific cleavage of DNA are studied and applied in various fields, such as DNA sequence determinations, chromosome analysis, gene therapeutics, and recombinant DNA manipulation[1]. Many sequence-specific cleavage reagents, which consist of transitional metals or polynuclear metal complex as "scissors" moiety and DNA as sequence-recognizing moiety, are developed. However, most of these "scissors", such as Fe^{2+}, Cu^{2+} and dinuclear iron (III) complex[2-6], which cleave DNA by free radicals, will break down DNA by oxidizing the ribose, thus cut fragments of the DNA target can not be further utilized. In addition, although Watson-Crick base pairing is remarkably specific, the mismatch discrimination of the DNA recognizer is not sufficiently selective, and more importantly, the DNA recognizer is susceptible to be hydrolyzed by endogenous nucleases and protease. Therefore, these kinds of reagents are unable to be applied *in vivo*. In the past few years, some rare earth ions as well as their complexes have been discovered to be effective in promoting hydrolysis of the phosphodiester linkage in DNA and the produced fragment has 3' and 5' glutinous terminus, with not damaged bases and ribose[7]. Recently detailed reports on cerium (IV) hydrolysis of oligomer DNA[8] showed that in all these ions, cerium (IV) and its complexes were especially prominent. PNA is a structural DNA mimic, which has a neutral polyamide backbone[9]. PNA has been reported to form Watson–Crick complementary duplexes with DNA or RNA[10]. In comparison with DNA/DNA duplexes, PNA/DNA duplexes offer several distinct advantages: greater stability than DNA

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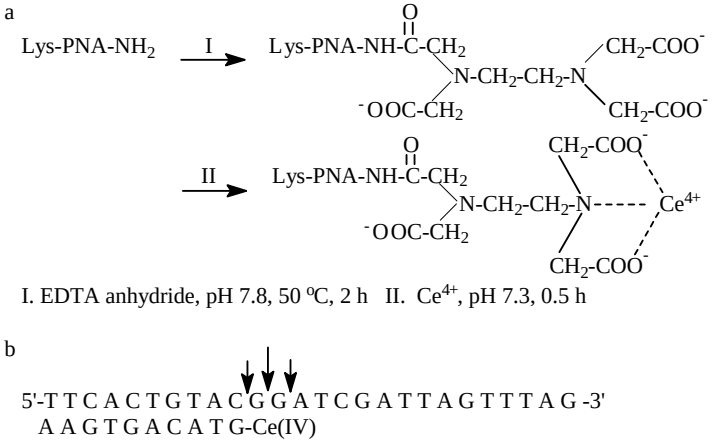
duplexes, and greater mismatch sensitivity. Furthermore, unlike the sugar-phosphate backbone, the polyamide one is immune to endogenous nucleases and protease. All these properties have exhibited the great superiority of PNA as potential DNA target recognition component.

Scheme 1

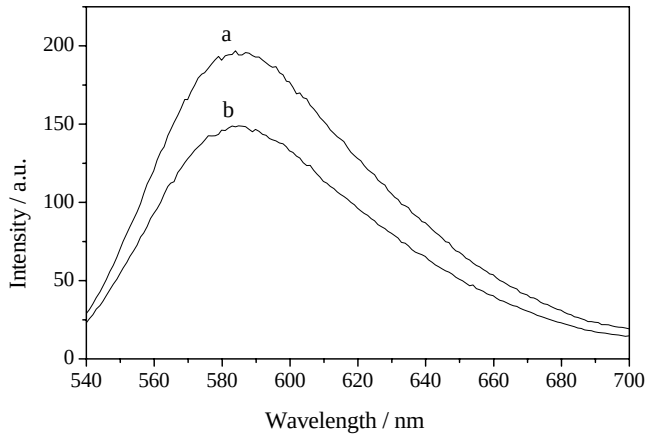


a. Synthesis of PNA-based artificial site specific cleavage reagent, b. Cleavage of target ssDNA by PNA-based artificial site specific cleavage reagent

Herein we developed a novel artificial site-specific cleavage reagent, which consisted of PNA as recognition unit to target DNA and cerium (IV) ions as scissors. The synthetic strategy is illustrated in **Scheme 1 (a),** and the application of it in site-specific hydrolytic cleavage of DNA is illustrated in **Scheme 1 (b)**.

The whole experiment procedure was as follows. 6 μL PNA (1 μmol/L) with an active amido at the N termini was reacted with 100 μL EDTA anhydride (0.04 mol/L) in 2 mol/L Tris-HCl buffer solution (pH 7.8) at 50 °C for 2 h, 247 μL cold ethanol-acetate buffer solution (pH 5.2) was then added. The mixture was stored at –20 °C overnight, and centrifuged at 10000 r/min for 15 min. The precipitate was washed with 70% ethanol, dried in air and dissolved in 8.5 μL 0.025 mol/L Tris-HCl buffer solution (pH 7.3). 1.0 μL target 26-mer ssDNA (0.2 μmol/L) were added to the obtained PNA-EDTA adducts solution. The mixture was incubated at 90 °C for 2 min, and slowly cooled to room temperature. The PNA adduct/target ssDNA duplexes, purified by the same method described above, were mixed with 10 μL 800 μmol/L Ce^{4+} in 2 mol/L Tris-HCl buffer solution (pH 7.3). The mixture was incubated at 37 °C for 48 h. The PNA-EDTA adducts were confirmed by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS). After reaction, two groups of peaks emerged in the spectrum, one of which corresponded to the original PNA (*m/z* 2904), and the other was assigned as the PNA-EDTA adducts (*m/z* 3176). This result indicated that PNA-EDTA adducts have been prepared, whereas some PNA remained unreacted.

Figure 1 Fluorescence spectra

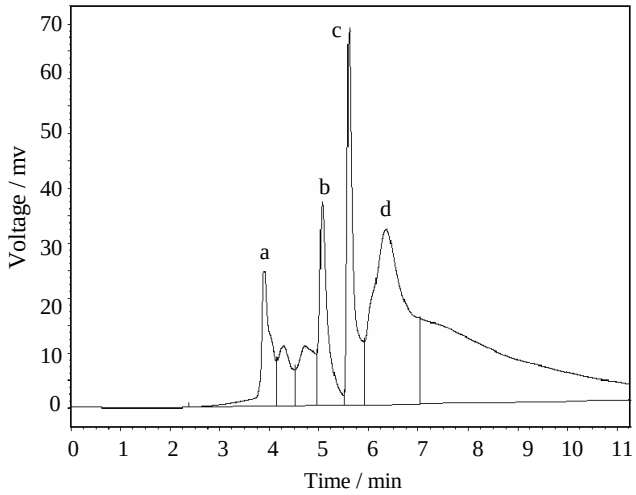


a. ST, b. ST interact with PNA-adduct/target ssDNA hybrids

Figure 1 is a fluorescence spectrum of the formation of PNA-adduct/target ssDNA hybrids. **Figure 1 (a)** is the fluorescence spectra of safranine T (ST). **Figure 1 (b)** is the fluorescence spectra of ST after interacting with PNA-adduct/target ssDNA hybrids, the fluorescence of the latter was quenched much. In addition, the formation of the hybrids was further confirmed by polyacrylamide gel electrophoresis. The result showed that the mobile rate of the target ssDNA and the hybrids was different in the gel. Due to the uncharged backbone of PNA and the steric effect, the hybrids migrate more slowly. The stability of the hybrids has also been measured by testing their dissociation temperature, which is about 85 ^{o}C. This dissociation temperature was much higher than that of the dsDNA. Therefore, it can be sure that the PNA-EDTA adducts can firmly hybridize with the target ssDNA.

According to the scission profile in **Scheme 1 (b)**, the scission should take place mostly at the linkage between G11 and G12. We can suppose that two kinds of products, that is, 15-mer ssDNA cut off specifically and the remained PNA-adduct/ 11-mer ssDNA hybrids would be yielded. The cleavage reaction was incomplete, so some PNA- adduct/target ssDNA hybrids existed. Moreover, in order to make the target ssDNA fully cleaved, excess artificial nuclease was added, thus the PNA-EDTA adduct was sure to be existed. **Figure 2** is the RP-HPLC chromatograms of the products of the cleavage reaction. The peak at 5.60 and 6.42 min are assigned to 15-mer ssDNA and the PNA-adduct/ target ssDNA hybrids, respectively. The peak at 3.85 min is corresponding to the PNA-EDTA adduct, while the peak at 5.07 min might be due to PNA-adduct/11-mer ssDNA hybrids. It can be concluded that the artificial site-specific cleavage regent has performed as expected.

Figure 2 RP-HPLC chromatograms



a. PNA-EDTA adduct, b. 15-mer ssDNA, c. PNA-EDTA/11-mer ssDNA hybrids, d. PNA-EDTA/target ssDNA hybrids

In summary, a novel PNA-based artificial site-specific cleavage reagent, which can selectively cleave target DNA at any desired site, was synthesized by conjugating cerium (IV) ions to PNA *via* EDTA ligands. It could provide a valuable tool to molecular biology and disease therapy.

Acknowledgments

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References and Notes

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