J|A|C|S COMMUNICATIONS

Published on Web 03/20/2007

A Sensitive and Homogeneous SNP Detection Using Cationic Conjugated Polymers

Xinrui Duan,[†] Zhengping Li,*[,†] Fang He,[‡] and Shu Wang*[,‡]

College of Chemistry and Environmental Science, Hebei University, Baoding 071002, Hebei Province, P. R. China, and Beijing National Laboratory for Molecular Sciences, Key Laboratory of Organic Solids, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100080, P. R. China

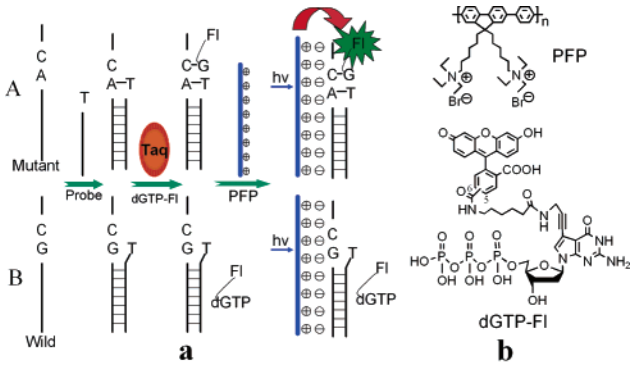
Received January 28, 2007; E-mail: lzpbd@mail.hbu.edu.cn; wangshu@iccas.ac.cn

As the human genome project has uncovered the full sequence of human genomes, the analysis of the variations among individual genomes, especially of single nucleotide polymorphisms (SNPs), has become an area of intense investigation for better understanding and treating the complex diseases of humans.[1,2] SNPs can be used as not only high-resolution genetic markers for mapping gene, defining population structure, and performing gene association studies[3] but also a fundamental tool for identifying numerous genetic and inherited diseases and drug discovery.[4,5] Various methods for SNP detection have been reported,[6,7] such as single-strand conformation polymorphism analysis, heteroduplex analysis, allele specific oligonucleotide hybridization, enzyme mismatch cleavage, oligonucleotide ligation, and invader assays. However, most of these methods are based on gel electrophoresis or a solid support phase to immobilize the oligonucleotide probes and, therefore, need the cumbersome protocols or multiple washing steps. As a way to circumvent the limitations, several techniques have been developed for detecting SNPs in homogeneous solution, such as fluorescence polarization (FP)[8] and fluorescence resonance energy transfer (FRET, e.g., TaqMan, molecular beacon).[9,10] The FP technique suffers the low sensitivity because of low polarized fluorescence intensity. Molecular beacons and TaqMan probes require doubly labeled DNA probes, which are expensive. There is thus a requirement for rapid, sensitive, and cost-effective methods for SNP detections.

Conjugated polymers (CPs) contain a large number of absorbing units, and the transfer of excitation energy along the whole backbone of the CP to the chromophore reporter results in the amplification of fluorescence signals. Therefore, CPs can be used as the optical platforms in highly sensitive chemical and biological sensors.[11−18] Microspheres, melting curves, or PNA probe-based SNP detection by using CPs have been developed; however, they need either multiple steps, sophisticated processes, or expensive PNA.[19] As demonstrated here, it is possible to use CPs to design a simple, sensitive, homogeneous, and rapid platform for SNP detection.

Our new assay strategy for SNP detection is illustrated in Scheme 1a. The target DNA fragment is part of p53 exon8 containing a polymorphic site (Arg282Trp),[20] where the nucleotide G in a wild-type target is replaced by A in a mutant. The 3′-terminal base of the probe is T that is complementary to the mutant-type target sequence and is not complementary to the wild-type target at the 3′-terminus (Scheme 1c). Taq DNA polymerase and dGTP labeled with a fluorescein (dGTP-Fl) at the guanine base (Scheme 1b) were used for probe extension reactions. Poly[(9,9-bis(6′-*N*,*N*,*N*-triethylammonium)hexyl)fluorenylene phenylene] (PFP, see structure in Scheme 1b)[14,18] is used as the cationic conjugated polymer in energy transfer experiments. The fluorescein label in dGTP, with

Scheme 1. (a) Schematic Representation of the SNP Assay; (b) Chemical Structures of PFP and dGTP-Fl; (c) DNA Sequences Used in the Assay: The Mismatched Base in the Mutant Target is Underlined



Wild target: 5′-CCTCTGTGCGCCGGTCTCTCCCAGGACAGGCA-3′
Mutant target: 5′-CCTCTGTGCGCCAGTCTCTCCCAGGACAGGCA-3′
Probe: 5′-TGCCTGTCCTGGGAGAGACT-3′

c

an absorption maximum at 480 nm, was chosen since its absorption overlaps with the emission of PFP with the emission maximum at 424 nm. Irradiation at 380 nm selectively excites PFP, and FRET from PFP (donor) to fluorescein (acceptor) is favored.[14] In situation A of Scheme 1a, the probe is fully complementary to the mutant target, and the dGTP-Fl is incorporated into the probe by extension reaction in the presence of Taq DNA polymerase. Upon adding the cationic conjugated polymer, strong electrostatic interactions between DNA and PFP bring the fluorescein close to PFP and efficient FRET from PFP to fluorescein occurs. In situation B, the 3′-terminal base of the probe is not complementary to the wild-type target, thus the base extension reactions cannot be performed, and the dGTP-Fl remains in the solution. In this case, upon adding the PFP, much weaker electrostatic interactions between the dGTP-Fl and PFP keep the fluorescein far away from PFP, and FRET from PFP to fluorescein is inefficient.

Figure 1a compares the emission spectra observed upon adding PFP ([PFP] = 1.5×10^{-6} M in monomer repeat units (RUs)) to probe extension solutions that were diluted to a mutant or wild-type target DNA concentration of 1×10^{-8} M with 200 mM phosphate buffered saline (PBS, pH 7.4). By addition of PFP, the comparison of the resulting fluorescence from fluorescein obtained by excitation at 380 nm shows an approximately 4 times higher signal for the complementary target/probe pair (mutant target and probe) extension product, relative to the one base mismatch wild target/probe pair. From the intensity changes of PFP emission, the FRET efficiency was measured to be 42% with the RSD of 3.7%. Note that, in comparison to the control experiment (wild-target with dGTP-Fl extension in Taq DNA polymerase reaction buffer without

[†] Hebei University.
[‡] Chinese Academy of Sciences.

10.1021/ja070452c CCC: $37.00

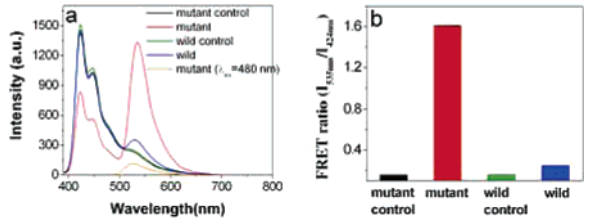


Figure 1. Emission spectra (a) and FRET ratio (I_{535nm}/I_{424nm}) (b) from solutions containing PFP and extension products of mutant and wild target DNAs in Taq DNA polymerase reaction buffer containing 1.5 mM Mg^{2+} ions. The extension solutions without Mg^{2+} ions are used as control. The probe extension solutions are diluted by 200 times with 200 mM PBS buffer (pH 7.4) before fluorescence measurements. The final concentration: [mutant or wild target] $= 1 \times 10^{-8}$ M, [mutant probe] $= 2 \times 10^{-8}$ M, [dGTP-Fl] $= 5 \times 10^{-8}$ M, [PFP] $= 1.5 \times 10^{-6}$ M in RUs. The excitation wavelength is 380 nm.

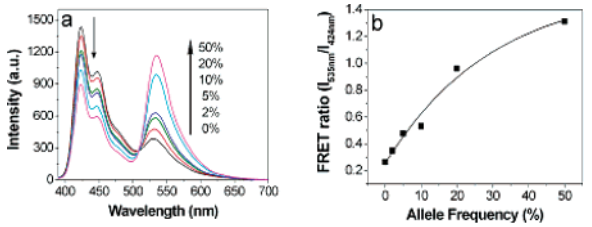


Figure 2. (a) Emission spectra of the extension production at various allele frequencies. (b) FRET ratio (I_{535nm}/I_{424nm}) as a function of allele frequencies. The total concentration of mutant and wild-type target is 1×10^{-8} M; [mutant probe] $= 2 \times 10^{-8}$ M, [dGTP-Fl] $= 5 \times 10^{-8}$ M, [PFP] $= 1.5 \times 10^{-6}$ M in RUs. The excitation wavelength is 380 nm.

Mg^{2+} ions), the wild target in the presence of Mg^{2+} ions demonstrates a little higher fluorescence signal of fluorescein. This indicates that a weak false-positive nonspecific extension reaction occurs.[20] However, the FRET ratio (I_{535nm}/I_{424nm}) for specific extension (mutant target and probe) is 8 times higher than that of the nonspecific extension (wild target and probe, Figure 1b), which demonstrates the good selectivity of this assay method for the SNP detection. Furthermore, the emission intensity of fluorescein using 380 nm excitation is approximately 10 times larger than that obtained by direct excitation at the fluorescein absorption maximum (480 nm) due to the optical amplification by the conjugated polymers. The increased fluorescein emission by the FRET imparts this SNP assay with high sensitivity.

The quantitative determination of the allele frequency has a important significance for the association study between SNPs and diseases.[20,21] To take advantage of selectivity and sensitivity that is obtained in our SNP assay method, we demonstrate the potential of the proposed assay for determination of the allele frequency by investigating the relationship between the allele frequencies and the FRET ratio (I_{535nm}/I_{424nm}) of extension production of test samples. Samples at various allele frequencies were composed of mutant-type target and wild-type target DNA fragments mixed at various ratios of 0, 0.02, 0.05, 0.1, 0.2, and 0.5. Total concentration of mutant and wild-type target is 1×10^{-8} M. Figure 2a shows the emission spectra of test samples. As the ratio of mutant target in the test sample increases, the emission intensity of fluorescein at 535 nm increases and that of PFP at 424 nm decreases. Relationship between the FRET ratio (I_{535nm}/I_{424nm}) and allele frequencies is shown in Figure 2b. It is noticeable that as low as 2% allele frequency was detected. In other words, the mutant-type target can be detected in the presence of the wild-type target, in which the concentration is 49 times higher than that of the mutant-type target.

In summary, we report a convenient and homogeneous fluorescence method for SNP detection as well as allele frequency determination with the light-harvesting properties of conjugated polymers. The higher FRET efficiency between PFP and dGTP-Fl is correlated to the incorporation of dGTP-Fl when target/probe pair is complementary at the polymorphic site. Taking advantage of the high specificity of the assay method, the mutant target can be determined in a mixed solution containing 49 times higher concentration of wild target. The allele frequency as low as 2% can be accurately determined with a common spectrofluorimeter in a homogeneous and cost-effective manner.

Acknowledgment. The project is supported by the National Natural Science Foundation of China (20675023, 20574073, and 20421101), the program for New Century Excellent Talents in University (NCET-05-0258), and the "100 Talents" program of the Chinese Academy of Sciences.

Supporting Information Available: Details for the fluorescence experiments, extension reactions, assays for allele frequencies, and complete ref 1. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Venter, J. C.; et al. *Science* **2001**, *291*, 1304−1351.
(2) Halushka, M. K.; Fan, J. B.; Bentley, K.; Hsie, L.; Shen, N.; Weder, A.; Cooper, R.; Lipshutz, R.; Chakravarti, A. *Nat. Genet.* **1999**, *22*, 239−247.
(3) Kuhner, M. K.; Beerli, P.; Yamato, J.; Felsenstein, J. *Genetics* **2000**, *156*, 439−447.
(4) Irizarry, K.; Kustanovich, V.; Li, C.; Brown, N.; Nelson, S.; Wong, W.; Lee, C. J. *Nat. Genet.* **2000**, *26*, 233−236.
(5) McCarthy, J. J.; Hilfiker, R. *Nat. Biotechnol.* **2000**, *18*, 505−508.
(6) Kirk, B. W.; Feinsod, M.; Favis, R.; Kliman, R. M.; Barany, F. *Nucleic Acids Res.* **2002**, *30*, 3295−3311.
(7) (a) Cotton, R. G. H.; Edkins, E.; Forrest, S. *Mutation Detection*; Oxford University Press Inc.: New York. (b) Chen, X. N.; Levine, L.; Kwok, P. Y. *Genome Res.* **1999**, *9*, 492−498.
(8) Tyagi, S.; Kramer, F. R. *Nat. Biotechnol.* **1996**, *14*, 303−308.
(9) Holland, P. M.; Abramson, R. D.; Watson, R.; Gelfand, D. H. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 7276−7280.
(10) McQuade, D. T.; Pullen, A. E.; Swager, T. M. *Chem. Rev.* **2000**, *100*, 2537−2574.
(11) Liu, B.; Bazan, G. C. *Chem. Mater.* **2004**, *16*, 4467−4476.
(12) Achyuthan, K. E.; Bergstedt, T. S.; Chen, L.; Jones, R. M.; Kumaraswamy, S.; Kushon, S. A.; Ley, K. D.; Lu, L.; McBranch, D.; Mukundan, H.; Rininsland, F.; Shi, X.; Xia, W.; Whitten, D. G. *J. Mater. Chem.* **2005**, *15*, 2648.
(13) Bunz, U. H. F. *Chem. Rev.* **2000**, *100*, 1605−1644.
(14) He, F.; Tang, Y.; Yu, M.; Feng, F.; An, L.; Sun, H.; Wang, S.; Li, Y.; Zhu, D.; Bazan, G. C. *J. Am. Chem. Soc.* **2006**, *128*, 6764−6765.
(15) He, F.; Tang, Y.; Wang, S.; Li, Y.; Zhu, D. *J. Am. Chem. Soc.* **2005**, *127*, 12343−12346.
(16) Nilsson, K. P. R.; Inganäs, O. *Nat. Mater.* **2003**, *2*, 419−424.
(17) Pinto, M. R.; Schanze, K. S. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 7505−7510.
(18) Gaylord, B. S.; Heeger, A. J.; Bazan, G. C. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 10954−10957.
(19) (a) Kushon, S. A.; Ley, K. D.; Bradford, K.; Jones, R. M.; McBranch, D.; Whitten, D. *Langmuir* **2002**, *18*, 7245−7249. (b) Gaylord, B. S.; Massie, M. R.; Feinstein, S. C.; Bazan, G. C. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 34−39. (c) Xu, H.; Wu, H.; Huang, F.; Song, S.; Li, W.; Cao, Y.; Fan, C. *Nucleic Acids Res.* **2005**, *33*, e83. (d) Tian, N.; Tang, Y.; Xu, Q.; Wang, S. *Macromol. Rapid Commun.* **2007**, *28*, 729−732.
(20) Li, Z. P.; Tsunoda, H.; Okano, K.; Nagai, K.; Kambara, H. *Anal. Chem.* **2003**, *75*, 3345−3351.
(21) Cermer, S.; Holland, M. J.; Higuchi, R. *Genome Res.* **2000**, *10*, 258−266.

JA070452C